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Isolation and morphological characterization of rice pathogens *Pyricularia* and *Bipolaris Oryzae* in metekal zone, north west Ethiopia

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Abstract
Rice blast disease, caused by *Pyricularia oryzae* Cavara is one of the major biotic factors and the most destructive of all rice diseases impeding rice production in more than 85 countries in the world. Brown spot disease of rice is caused by *Bipolaris oryzae* (Breda de Haan) Shoemaker, (Teleomorph – Cochliobolus miyabeanus) was major problem eventually caused sustainable losses both in quality and quantity. The present study aimed to characterize morphologically rice blast and brown spot diseases. Zones and districts were selected purposely based on rice area coverage at heading to dough crop growth stage. The samples were collected from 72 farmers' fields of rice and six localities in each two districts. It was conducted during mid-October to mid November 2020/2021 main cropping season in Pawe and Jawi districts. The fungus, *P. oryzae* and *B. oryzae* were isolated from single discrete lesion from the infected leaf tissue or panicle. Commercial potato dextrose agar (PDA) medium was used for isolation. Bacterial growth/contamination was avoided by incorporating antibiotic chloramphenicol in the media. The growing mycelium were purified and replicated two times on PDA by exploiting the principle of completed randomized design (CRD). The isolates were characterized based on the morphological characteristics of the pathogen with the help of keys by Barnett and Hunter. The results revealed that, there was a considerable significant variation among the isolates of *P. oryzae* and *B. oryzae* in colony radial growth, conidial length and conidial width on PDA media. From the total of 45 *P. oryzae* and 32 *B. oryzae* isolates, 15 and 11 isolates, respectively were shown morphological variations.

Keywords: *Bipolaris oryzae*, characterization, isolation, *Pyricularia oryzae* and rice

Introduction
Rice (*Oryza sativa* L. and *Oryza glaberrima* Steud.) is central to the lives of billions of people around the world. Rice is the oldest domesticated grain serving as cereal grain. It is major food crop that is staple for more than half of the world's human population, especially in Asia. About 3.5 billion people depend on rice as a daily food staple for 20 % of their calories [1]. Worldwide, rice was grown in an area of 165.2 million ha with a total production of 741.0 million tons [2]. This production could not satisfy the existing demand of rice. Accordingly, yields must be doubled by the next three decades to sustain the nutritional need of the ever-expanding global population [3, 4].

In Africa rice is the fastest growing source of food. During the past three decades, rice grain has seen a steady increase in consumption and demand given its important place in the strategic food security planning policies of many African countries [5, 6].

In Ethiopia according to the report of [7] the potential rice production area is estimated to be about 30 million hectares, of which more than 5 million ha is highly suitable. According to CSA [8] in 2017 & 2018 total production has increased by 13.80%, but productivity in 2019 reduced by 4.63% t/ha due to different biotic and abiotic factors.

In the previous, numerous diseases were reported in rice crop. The major diseases were blast (*Pyricularia oryzae* Cavara), bacterial blight, sheath rot (*Sarocladium oryzae*), sheath blight (*Rizhoctonia solani*) and brown spot (*Bipolaris oryzae*) [9]. Rice blast disease, caused by *Pyricularia oryzae* Cavara (teleomorph *Magnaporthe grisea* (Hebert) Barr), is one of the biotic factor and the most destructive of all rice diseases impeding rice production in more than 85 countries in the world [10]. It is a worldwide problem in rice and is dangerous because of its yield loss potential ranging up to 100% under favorable conditions [11]. Brown spot disease of rice is caused by *Bipolaris oryzae* (Breda de Haan) Shoemaker, (Teleomorph – *Cochliobolus miyabeanus*) was major problem eventually caused sustainable losses both in quality and quantity [12]. It has both an asexual and a sexual stage. The asexual stage is *Bipolaris oryzae* (Breda de Haan) Shoemaker [13]. The sexual stage is *Cochliobolus miyabeanus* (Ito and Kurib) Drechsler ex Dastur. Nectrophs like Bipolaris oryzae kills the host by producing toxin and lytic enzymes [14]. It reduced rice yield from 50% to 90% in Bengal [15, 16].

In Ethiopia [15], have been reported that panicle blast and brown spot were more severe diseases on upland rice. Even though frequent occurrence of epidemics of blast and brown spot diseases in the rice growing areas of the country, there is no any information on isolation and morphological characterization of the diseases in Pawe and

Jawi districts as of today. Therefore this study was initiated to characterize morphologically rice blast and brown spot diseases.

Materials and Methods

Description of the Study Area

Sample collection was conducted during mid-October to mid November 2020/2021 main cropping season in Pawe and Jawi districts of Metakal and Awi zone of Benishangul Gumuz and Amhara Regional state, respectively. Pawe is located 575 Km apart from Addis Abeba. Its geographical location is between11^0 15'and 11^0 23' north latitude and 36^0 30'east longitude at an altitude of 1120 meters above sea level (Figure 5). The mean annual temperature ranges from 16^0C to 32^0C. Pawe is characterized as a lowland area with very high rainfall (hot humid area) and has unimodal pattern with extended rainy season from May to October, having mean annual rain fall of 1661 mm per year. Its average humidity is 54.7%, with a wind speed of 53.6 km/day and sunshine hours of 7.75. It has three types of soils, namely Vertisol which account for 40-45%, Nitosol which accounts 25-30% and intermediate soil of Luvisol which account for 25-30% of the total of the center [18].

Sample Size and Collection

Zones and districts were selected purposely based on rice area coverage at heading to dough crop growth stage. The samples were collected from 72 farmers' fields of rice and six localities in each two districts. Totally, 72 samples for blast and 72 for brown spot pathogens were collected for isolation of both pathogens. Blast and brown from infected rice leaf, sheath, panicle and seed samples at booting to dough growth stage were collected from farmer's fields at an altitude ranges between 1027 and 1310m.a.s.l. Infected plant parts were cut from the mother plant and placed in a paper bag, which were labeled with all necessary information including the name of the region, zone, district, kebeles, and name of the cultivar, GPS data and date of collection. Samples were kept in refrigerator at 5ºC until the field assessments in both districts were completed. Then, the samples were preserved in ice box and transported to Ambo Agricultural Research Center (AmARC) for pathogen isolation, identification and characterization at plant mycology laboratory.

Pathogens Isolation and Characterization

The fungus, *P. oryzae*, and *B. oryzae* were isolated from single discrete lesion from the infected leaf tissue or panicle. Infected plant parts were cut into small pieces (less than 1.0 cm in size) around the area showing the lesions including the edge of the lesion and were surface sterilized with 1% sodium hypochlorite for 1 minute followed by 3 washes with sterile distilled water and placed on Petri dishes lined with moist filter papers and then by using a sterile moistened needle, five (5) infected plant pieces were transferred on to a sterilized petri dishes containing commercial potato dextrose agar (PDA) medium (Figure 3). To avoid bacterial growth/contamination antibiotic chloramphenicol at the rate of 2 capsule/litter was incorporated in the media [19].
It was incubated for 3-5 days and further mycelial growth was sub cultured and incubated at 27°C ±2 for 5 to 10 days to induce sporulation of the fungi [20]. The growing mycelium were purified and replicated two times on PDA by exploiting the principle completed randomized design (CRD). The isolates were characterized based on the morphological characteristics of the pathogen with the help of keys by Barnett and Hunter [21].
The mycelium color of each isolate on PDA medium, were examined at ten days of incubation. The colony diameter of each isolate on Petri plates was measured in two directions with a digital electronic caliper at three days intervals and the measurements were recorded in centimeter (cm). Type of margin, surface texture and aerial mycelium were also recorded according to Barnett and Hunter [22], procedure.
A small portion of the growing mycelium from each isolate were prepared on slides by using lacto phenol cotton blue and observed under light microscope at 10 and 40X for confirmation of the rice blast and brown spot isolates (Figure 3). The shapes of the conidia and conidiophore were determined as described by Meena [23].

Result and Discussions

Pyricularia Oryzae

The results revealed that there was a considerable significant variation among the isolates of *P. o*ryzae in colony radial growth, conidial length and conidial width on PDA media (Table 1). From the total of 45 *P. oryzae* isolates, 15 of them were shown morphological variations.

Table 1: ANOVA table for colony radial growth, conidial length and conidial width of *P. oryzae* isolates of Pawe and Jawi in 2020/2021

Sources of variations	Rep (df=1)	Trt (df=14)	Error (df=14)	C.V	Mean
CRG (cm)	0.35	6.31***	0.15	8.7	4.49
CL (µm)	1.83	14.89***	0.09	1.3	23.18
CW (µm)	0.73	0.74***	0.007	1.13	7.8

**Rep = replication, Trt = Treatments, C.V = coefficient of variation, CRG = colony radial growth, CL= conidial length and CW = conidial width.

The means of radial growth of 15 isolates of *P. oryzae* on PDA were shown significant variations. The maximum colony radial growth (7.95cm) was recorded on isolate *Po*15/756A and *Po*15/756C followed by *Po*15/7756C (6.40cm) and their difference was statistically significant (Table 2). However, the minimum radial growth (1.83cm) was observed on isolate *Po*4/744A. Similar results were also reported by [24], there was a considerable significant variation among the colony diameter of the *P. oryzae* isolates on PDA media. The radial growth rates were varying among isolates depending on the origin of the sample, altitude difference and sexual stage. The colors of isolates were varied from dark brown were white greyish. *Po*15/756A, *Po*15/756B, *Po*15/756C, *Po*62/798A, *Po*17/758B and *Po*49/776A were white in color whereas, *Po*9/750, *Po*17/758A, *Po*9/750, *Po*56/779 and *Po*14/758A were dark brown in color (Figure 1).

Most of *P. oryzae* isolates were entire in margin, except *Po*9/750B, *Po*14/755B and *Po*3/743 which were irregular in margin. *Po*15/756A, *Po*14/755A, *Po*15/756C, *Po*2/742, *Po*3/743, *Po*9/750, *Po*17/758A and *Po*14/755B isolates were shown thick fluffy (heavy cottony) surface texture and *Po*62/798, *Po*56/779A, and *Po*5/745 were shown uncompressed fluffy. However, *Po*15/756B, *Po*56/779B, *Po*4/744A, *Po*17/758B, *Po*14/755B, *Po*49/776A, *Po*2/742, *Po*59/793 and *Po*47/771 were shown velvety (smooth) surface texture.

The means of conidial length and width were also shown significant variation among isolates of *P. oryzae*. The highest conidial length and width in micro meter (µm) were recorded by *Po*56/779A which is 27.40 and 9.20µm, respectively. However, the lowest conidial length and width were recorded by *Po*4/744A which is 18.50 and 5.60µm, respectively. And also, the results showed that in most *P. oryzae* isolates, the shape of the conidia was typically pyriform with base rounded, apex narrowed, 2-3 septate, 2- 4 celled (Figure 1).

Table 2: Mean separation for colony radial growth, conidial length, conidial width and mycelium features of *P. oryzae* isolates of Pawe and Jawi in 2020/2021

Isolates	Location	Variety	Plant part	CRG (cm)	CL (µm)	CW (µm)	Mycelium Features
*Po*15/756A	Pawe	Gumara	Leaf	7.95^{a}	24.25^{d}	7.25^{f}	White, thick fluffy
*Po*14/755A	Pawe	NERICA 13	Leaf	4.20^{de}	22.05^{gf}	6.35^{h}	Dark brown, thick fluffy
*Po*15/756C	Pawe	NERICA 13	Panicle	7.95^{a}	23.35^{e}	7.60^{e}	White, thick fluffy
*Po*15/756B	Pawe	NERICA-4	Panicle	6.4^{b}	27.30^{a}	8.60^{b}	White, velvety
*Po*52/742	Jawi	NERICA-4	Panicle	4.05^{de}	26.40^{b}	7.30^{f}	Black, velvety
*Po*16/758B	Jawi	NERICA-4	Panicle	3.77^{fe}	23.90^{ed}	8.35^{c}	White greyish, uncompact fluffy
*Po*56/779B	Pawe	Local	Leaf	4.71^{dc}	22.25^{f}	7.65^{e}	Dark brown, velvety
*Po*4/744A	Jawi	Hidasse	Seed	1.83^{g}	18.50^{i}	5.60^{i}	White greyish, velvety
*Po*17/758B	Pawe	Hidasse	Leaf	3.20^{f}	21.60^{g}	7.35^{f}	White, velvety
*Po*14/755B	Pawe	Local	Leaf	5.45^{c}	25.35^{c}	8.25^{c}	D/brown, velvety
*Po*56/779A	Pawe	Local	Leaf	3.57^{fe}	27.40^{a}	9.20^{a}	Greyish, uncompact cottony
*Po*62/798A	Pawe	Local	Leaf	3.63^{fe}	20.80^{h}	7.40^{f}	W/greyish, uncompact cottony
*Po*49/776A	Pawe	Local	Node	3.00^{f}	21.85^{gf}	6.60^{g}	White, velvety
*Po*9/750	Jawi	Local	Panicle	3.02^{f}	18.90^{i}	5.50^{i}	Dark brown, thick fluffy
*Po*17/758	Pawe	NERICA 13	Leaf	4.72^{dc}	23.80^{ed}	7.95^{d}	Dark brawn, thick fluffy
Mean				4.49	23.18	7.8	
LSD				0.84	0.65	0.18	
C.V				8.7	1.3	1.13	

***P.o =Pyricularia oryzae*, CRG= colony radial growth, CL=conidial length, CW= conidial width and µm= micro meter.

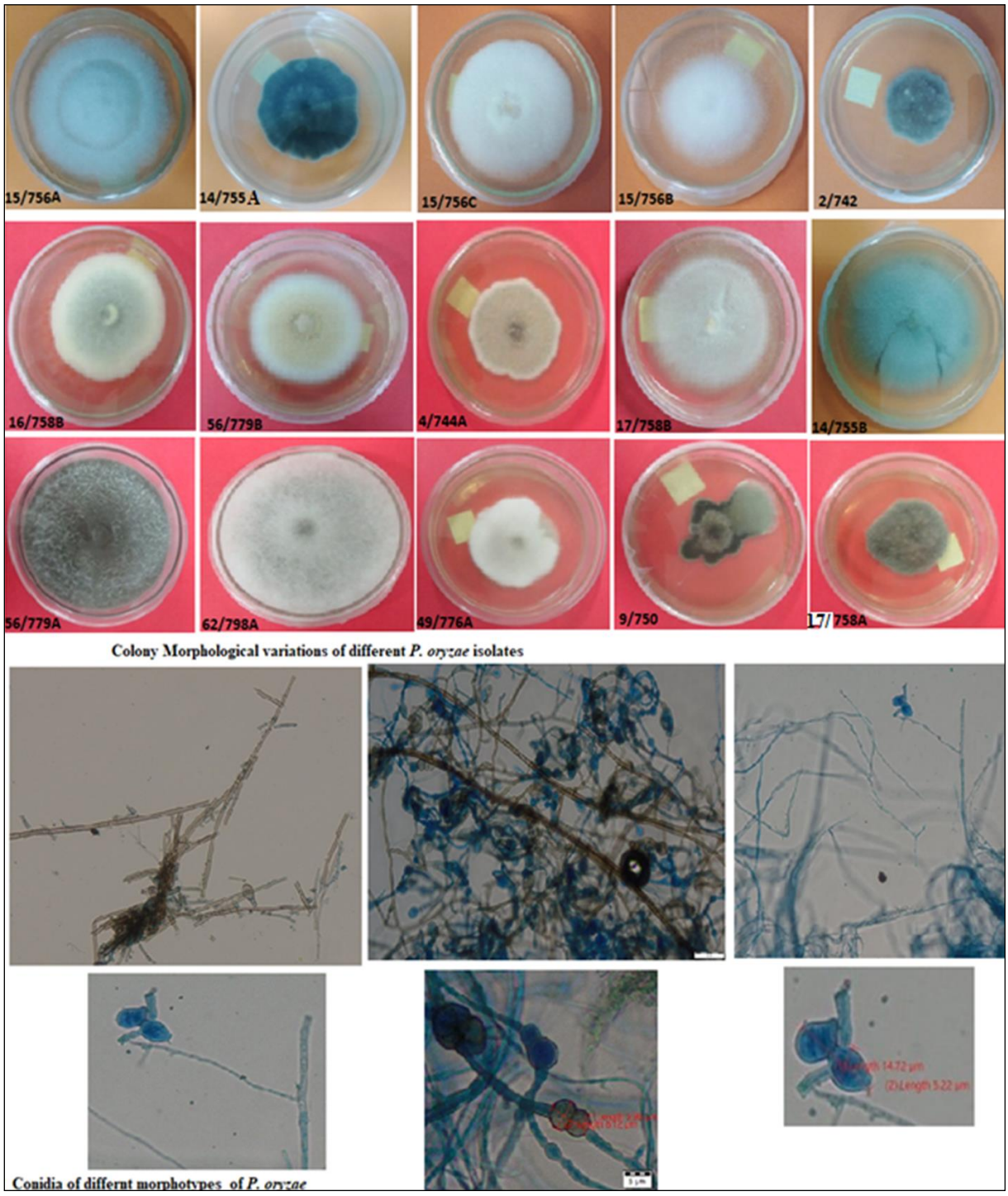

Fig 1: Colony and conidial morphological variations of *P. oryzae* isolates from Pawe and Jawi

Bipolaris oryzae

The results revealed that there is a significant variation among the colony radial growth, conidial length and width of the *B. oryzae* isolates on PDA media (Table 3). From the total of 38 *B. oryzae* isolates, 11 of them were shown morphological variations. More *B. oryzae* isolates were shown variations in mycelia shape and growth margin.

Table 3: ANOVA table for colony radial growth, conidial length and conidial width of *B.* oryzae isolates of Pawe and Jawi in 2020/2021

Sources variations	Rep (df=1)	Trt (df=10)	Error (df=10)	C.V	Mean
CRG (cm)	0.06	4.58***	0.14	10.48	3.55
CL (µm)	4.28	4.69**	0.95	3.27	29.79
CW (µm)	0.09	0.66	0.65	6.04	13.32

**Rep = replication, Trt = Treatments, C.V = coefficient of variation, CRG = colony radial growth, CL= conidial length and CW = conidial width.

The means of radial growth of 11 isolates of *P. oryzae* on PDA were shown significant variations. The maximum colony radial growth was observed on isolate *Bo*13/759B (6.35cm) followed by *Bo*13/758B (5.90cm) and their difference were statistically not significant. However, the minimum radial growth was observed *Bo*9/750B (1.07cm) followed by *Bo*14/744C (2.87) and their difference was statistically significant. *Bo*13/759C (3.25cm), *Bo*7/748B (3.05cm), *Bo*5/746 (3.05cm) and *Bo*50/777B (3.03cm) were shown medium growth and their differences were statistically not significant (Table 4).

The result also revealed that, *B. oryzae* isolates were varied in conidial length and width in micro meter. But, there is no significant different among *B. oryzae* isolates in conidial width. The longest conidial length (31.8µm) was recorded on *Bo*13/759B followed by *Bo*4/744C (31.50µm). However, the shorter conidial length (27.20 µm) was recorded by *Bo*62/798.

The *B. oryzae* isolates were varied in colony color. *Bo*50/777B was unique in colony color which is yellowish (Figure 2). [25], also reported that cultural characters (blackish, grey, white color mycelium with fluffy or cottony growth. Fajolu, [26] also reported that *B. oryzae* isolates within and between species varied in colonial and conidial morphology.

Most of *B. oryzae* isolates were even in margin, except *Bo*9/750B and *Bo*50/777B which were irregular in margin. Microscopically, *B. oryzae* isolate revealed the presence of single conidiophores, straight to flexuous and pale to brown in color. Conidia were slightly curved and widest at the middle, obclavate, 5 to 9 septate, cylindrical and pale to golden brown which was strongly agreed with the finding of [27] that Conidia were slightly curved and widest at the middle, obclavate, 5 to 10 septate, cylindrical and pale to golden brown. These results were also, in agreement with [28] who reported that, mycelium appeared to be grey to dark greenish grey and conidia were dark brown to olivaceous brown, straight or curved with 6-14 septation.

Table 4: Mean separation for colony radial growth, conidial length, conidial width and mycelium features of *B. oryzae* isolates of Pawe and Jawi in 2020/2021

S/No.	*B.o* isolates	Location	Variety	Plant part	CRG (cm)	CL(µm)	CW(µm)	Mycelium Features
1	*Bo*13/759B	Pawe	NERICA 13	Leaf	5.90^{a}	31.80^{a}	13.6	Brown, fluffy
2	*Bo*7/748A	Pawe	SUPERICA 1	Leaf	4.65^{b}	28.40^{fde}	13.1	Pink, smooth creamy
3	*Bo*13/759C	Pawe	NERICA 13	Panicle	3.25^{c}	30.95^{ba}	13.5	Dark white, creamy
4	*Bo*7/748B	Jawi	Local	Leaf	3.05^{c}	29.50^{bde}	13.85	Dark white, creamy
5	*Bo*13/759A	Jawi	Local	Leaf	6.35^{a}	30.06^{bac}	14.65	Dark brown, creamy
6	*Bo* 5/746	Pawe	Gumara	Leaf	3.05^{c}	27.95^{fe}	12.75	Greyish, smooth creamy
7	*Bo*62/798	Jawi	Kokit	Leaf	2.91^{c}	27.20^{f}	13.25	Dark brown, thick cottony
8	*Bo*9/750B	Pawe	Local	Leaf	1.07^{c}	30.80^{bac}	12.7	Greyish, smooth creamy
9	*Bo*4/744C	Jawi	Hidasse	Seed	2.87^{c}	31.50^{ba}	13.15	White, smooth
10	*Bo*49/776A	Jawi	Hidasse	Leaf	2.92^{c}	30.25^{bdac}	13.2	White brown, fluffy
11	*Bo*50/777B	Jawi	NERICA 4	Panicle	3.03^{c}	28.70^{fdec}	12.75	Black, fluffy
	Mean				3.55	29.79	13.32	
	L.S.D at 0.05				0.83	2.17	1.79	
	C.V (%)				10.48	3.27	6.04	

*******B.o =Bipolaris oryzae*, CRG= colony radial growth, CL=conidial length, CW= conidial width and µm= micro meter

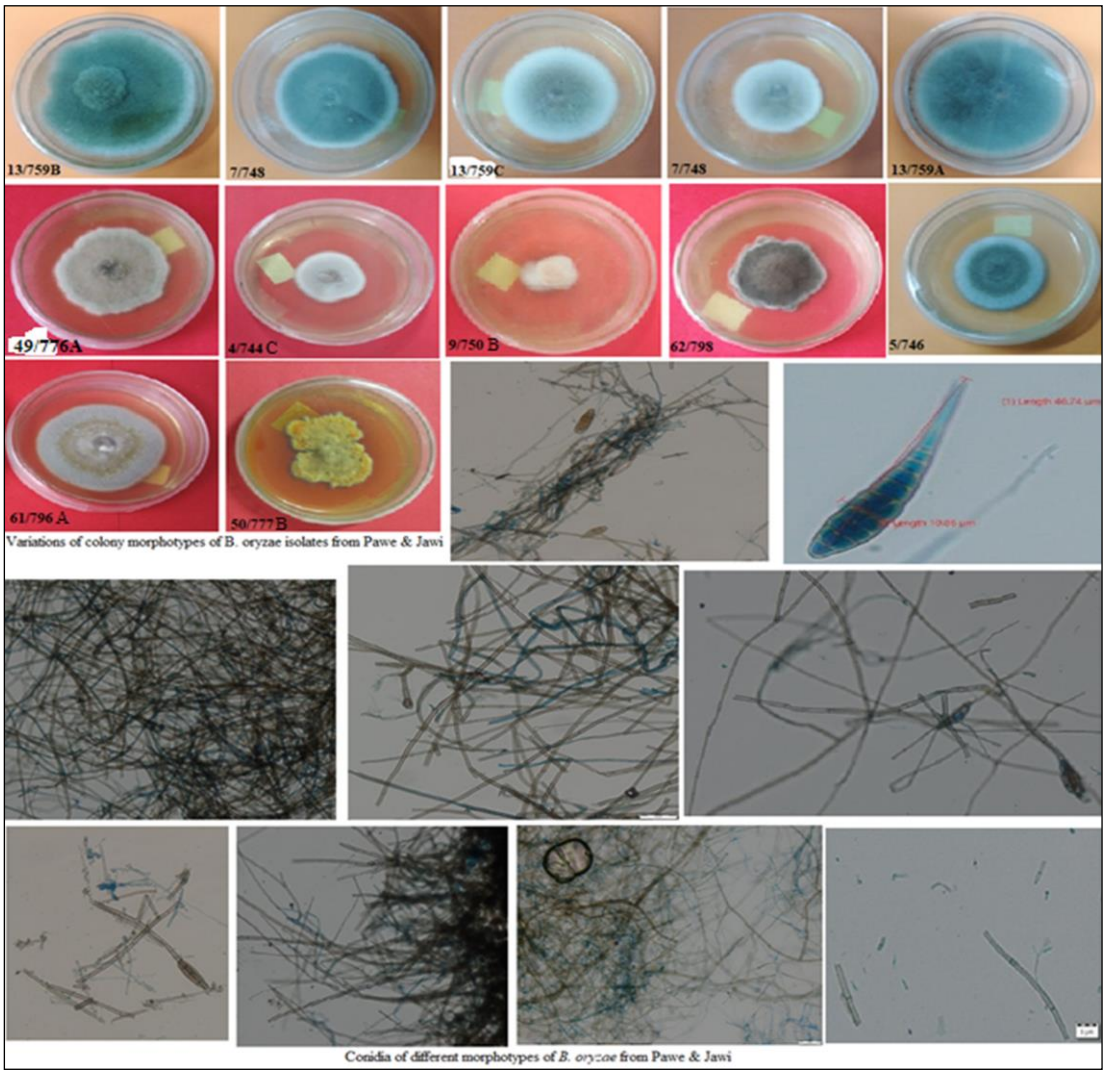

Fig 2: Morphotype variations in colonial and conidial character of *B. oryzae* isolates.

Fig 3: Overall laboratory activities: (A) Isolation, (B) Incubation, (C) Grouping of morphologically similar isolates (D) Identification (E) image capturing under microscope and (F) Preservation of purified isolates for future work

Conclusion

The results revealed that, there were variations in colony radial growth, surface appearance, colony color and texture among isolates of P. oryzae on PDA media and the same is true for both B. oryzae. Most isolates of *P.* oryzae and *B.* oryzae were shown even margin. The radial growth rates and colony color were varying among isolates of P. oryzae and B. oryzae depending on the origin of the sample, sexual stage and altitude difference.

For the future, intensive and extensive survey and surveillance be carried out across the major rice growing at different agro ecologies and rice ecology (upland, lowland and irrigated rice) in the country to develop disease distribution map and generate clear picture of the disease status and magnitude of losses due to major rice diseases. And also, go for further studies on species diversity, race analysis, and intensive morphological and molecular characterization of *P. oryzae* and *B. oryzae* fungal diseases of rice is the critical future emphasis of rice protection researchers of the country.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

1. OLAMI. Game-changing rice production methodology awarded olam prize for innovation in food security. News release, Singapour, 2015.
2. FAOSTAT. FAOSTAT Database, Statistic Division, Food and Agriculture Organisation of the United Nations, 2018.
3. Nalley L, Tsiboe F, Durand-Morat A, Shew A, Thoma G. Economic and environmental impact of rice blast pathogen (Magnaporthe oryzae) Alleviation in the United States. PLOS ONE, 2016, 11(12). e0167295. https://doi.org/10.1371/journal.pone.0167295.
4. Skamnioti P, Gurr SJ. Against the grain: Safeguarding rice from rice blast disease. Trends in Biotech,2009:27(3):141-150.
5. ARC. Africa rice trend. An overview of recent developments in Sub-Saharan Africa rice sector. Africa rice Center, Cotonou, Benin, 2007.
6. FARA. Patterns of change in rice production in Africa. Implications for rice policy development. Ministerial Policy Brief Series Number 2, Accra, Ghana, 2009, 1-7.
7. Ministry of Agriculture (MoA). National Rice Research and Development Strategy of Ethiopia. Addis Ababa, Ethiopia, 2010, 48.
8. CSA (Central Statistical Agency). Agricultural sample survey Report on area and Pro- duction of major crops. Central Statistical Agency of Ethiopia, Addis Ababa, Ethiopia. V.1. Statistical bulletin, 2019, 532.
9. Kindo D, Tiwari P. Efficacy of fungicides for management of sheath rot disease in rice under field conditions. Plant Archives,2015:15(1):119-120.
10. Katsantonis D, Kadoglidou K, Dramalis C, Puigdollers P. Rice blast forecasting models and their practical value: a review. Phytopathologia Mediterranea,2017:56(2):187-216. https://doi.org/10.14601/Phytopathol.
11. Netam RS, Bahadur AN, Tiwari U, Tiwari RKS. Efficacy of plant extracts for the control of (Pyricularia grisea) blast of rice under field condition of Bastar, Chattisgarh. Research Journal ofAgricultural Science,2011:2(2):269-271.
12. Ou SH. Rice Diseases, 2nd ed. Commonwealth agricultural bureau international, Kew, UK, 1985, 380.
13. Dela Paz MA. Molecular characterization of isolates causing brown spot of rice (Oryza sativa L.) in rainfed ecosystems in the Philippines. M.S. Thesis. College of Arts and Science. University of the Philippines at Los Baños, 2005.
14. Kan J, Van AL. Licensed to kill: the lifestyle of a necrotrophic plant pathogen. Trends in Plant Science,2006:11(5):247-253.
15. Padmanabhan SY. Estimating losses from rice blast in India. In the rice blast disease: Johan Hopkins Press, Baltimore, Maryland, 1965, 203-221.
16. Thuy TT. Infection biology of Bipolaris oryzae in rice and its pathogenic variation in the Mekong Delta, Vietnam. Ph.D. Dissertation. Department of plant biology, plant pathology section, The royal veterinary and agricultural university, Copenhagen, Denmark, 2002.

17. Jember Mulugeta Bitew, Firew Mekbib, Alemayehu Assefa. Genetic variability among yield and yield related traits in selected upland rice (Oryza sativa L. and Oryza glaberrima Steud) genotypes in Northwestern Ethiopia. WSN,2016:47(2):62-74.
18. EIAR (Ethiopian Institute of Agricultural Research). Soil classification and mapping of the centers, 2016.
19. Motlagh SMR, Zamanizadeh HR, Hedjaraude GHA, Okhovvat M. Identification of the causal agent fungi of rice brown spot disease in. Journal of Agricultural Scence and Naturural Resourses,2006;12:136-45.
20. Aneja KR. Experiments in Microbiology Plant Pathology and Biotechnology, New Age International Publishers, New Delhi, 2005.
21. Barnett HL, HBB. Illustrated Genera of Imperfecti Fungi, Burgess Publishing Company, West Virginia, 1998.
22. Huang B, Xu JY, Hou MS, Ali J, Mou TM. Introgression of bacterial blight resistance genes Xa7, Xa21, Xa22 and Xa23 into hybrid rice restorer lines by molecular marker-assisted selection. Euphytica,2012:187:449-459.
23. Meena BS. Morphological and Molecular Variability of Rice Blast Pathogen Pyricularia oryzae (Cooke) Sacc, Dharwad University of Agricultural Sciences, Dharwad, 2005.
24. Mebratu Gebremariam Asfaha, Thangavel Selvaraj, Getaneh Woldeab. Assessment of disease intensity and isolates characterization of blast disease (Pyricularia oryzae CAV.) from South West of Ethiopia. International J. of Life Sciences,2015:3(4):271-286.
25. Kumari S, Kumar A, Rani S. Morphological characterization of Bipolaris oryzae causing brown spot of paddy in Bihar. Int. Educ. Res. J.,2015:1(5):85-87.
26. Fajolu Oluseyi Lydia. "Characterization of Bipolaris species, their effects on switchgrass biomass yield and chemical components." PhD diss., University of Tennessee, 2012. https://trace.tennessee.edu/utk_graddiss/1581.
27. Sobanbabu G, Sabarinathan KG, Parthiban VK, Ramamoorthy V. Isolation, screening and identification of virulent isolates of Bipolaris oryzae causing rice brown spot and Sarocladium oryzae causing sheath rot disease. Int. J. Curr. Microbiol. App.Sci,2018:7(09):930-939. doi: https://doi.org/10.20546/ijcmas.709.112
28. Arshad HMI, Hussain N, AliS Khan JA, Saleem K, Babar MM. Behavior of Bipolaris oryzae at different temperatures, culture media. Pak J Phytopathol, 2013, 16(3).