RESEARCH ARTICLE

Assessment of disease intensity and isolates characterization of blast disease (*Pyricularia oryzae* CAV.) from South West of Ethiopia

Mebratu Gebremariam Asfaha[1], Thangavel Selvaraj[1]*and Getaneh Woldeab[2]

[1]Department of Plant Sciences, College of Agriculture and Veterinary Sciences, Ambo University, Ambo, Post Box No: 19, Ethiopia, East Africa.

[2]Department of Pathology, Ambo Plant Protection Research Center (APPRC), Ambo, Ethiopia, East Africa.
*Corresponding author: Email: tselvaraj_1956@yahoo.com: Mobile Number: +251-913073294.

Manuscript details:

Received: 23.10.2015
Revised: 20.11.2015
Accepted: 06.12.2015
Published : 30.12.2015

Editor: Dr. Arvind Chavhan

Cite this article as:
Mebratu Gebremariam Asfaha, Thangavel Selvaraj and Getaneh Woldeab (2015) Assessment of disease intensity and isolates characterization of blast disease (*Pyricularia oryzae* CAV.) from South West of Ethiopia. *International J. of Life Sciences*, 3(4): 271-286.

ABSTRACT

Blast (*Pyricularia oryzae* Cav.) disease is one of the most important biotic constraints to rice production in South West of Ethiopia. The present study was conducted to determine the intensity of the blast disease and their isolates characterization in upland rice cultivated fields of South West of SNNPRS of Ethiopia. The assessment of rice blast was carried out in 90 farmers' fields in three districts during the main cropping season from May to October 2013, in the major upland rice growing areas of Kaffa, Benchi-Maji and Sheka zones in South West of Ethiopia. The results of the assessment revealed that the incidence and severity of the disease vary from low to high on the rice fields depending on the agro-ecological and cultivars differences. Rice blast was observed in all assessed locations at variable levels. The incidence of rice blast in six different localities varied from 42.01 to 85.69%. The highest mean incidence of rice blast was recorded in Otuwa locality (85.69%) and the lowest incidence recorded in Argoba locality (42.01%). The overall mean incidence of six localities in three districts of the South west of Ethiopia (SNNPRS) reached 65.68%. Likewise, blast severity showed similar trend as that of incidence in all six localities. The highest severity was recorded in Otuwa locality with range of 8.88 - 88.8 % and the mean severity values of 55.7% while the lowest severity was recorded in Argoba locality (33.62%). The overall mean severity of the six localities in three districts of the South west SNNPRS reached 47.15%. The entire assessed three districts showed 100 % blast disease prevalence. Rice blast, *Po* isolates were characterized and identified based on their growth parameters into six isolates such as *Po*12, *Po*28, *Po*41, Po55, *Po*72 and *Po*85. Among the four culture media (oat meal agar, rice flour agar, malt extract agar and potato dextrose agar), the *Po* isolates were grown on optimum growth and good sporulation in oat meal agar

followed by rice flour agar. The optimum temperature and pH of the growth of the *Po* isolates were at 30°C and 6.5, in almost all isolates, respectively. It could be concluded that the rice blast was the most important disease of rice cultivars in South West (SNNPRS) of Ethiopia. Further studies could be conducted to evaluate the upland rice cultivars resistance against the isolated blast (*Po* isolates) pathogens under greenhouse and natural environmental conditions.

Key words: Rice, blast, *Pyricularia oryzae*, Assessment, Disease Intensity, *Po* isolates, Characterization.

INTRODUCTION

Rice (*Oryzae sativa* L.) belongs to the family Poaceae, is one of the main cereal food crops in most part of Africa, however, the rice cultivation and utilization as a food crop in Ethiopia is very recent phenomenon (Traore *et al.,* 2006; Abdu *et al.*, 2013). It is recognized as an important strategic food security crop and as a crucial element in the staple food economies of Sub Saharan Africa (Abdu *et al.*, 2013). It is also the most rapidly growing source of food crop in Africa, and significant importance to food security and food self-sufficiency in an increasing number of low-income food deficit countries (FAO, 2012). Rice cultivation in Ethiopia has begun at Fogera (Amara Region) and Gambella plains in the early 1970's (EIAR/ FRG II, 2012). It is reported that the potential rice production area in Ethiopia, which is estimated to be about thirty million hectares (MoARD, 2010). The number of farmers engaged in rice production has increased from about 53,302 ha in 2006 to about 284,868 ha in 2009. Similarly, the area allocated has increased from about 18,527 ha in 2006 to about 155,886 ha in 2009 along with production increase from about 42,825 tons in 2006 to about 498,332 tons in 2009. There is an increased trend in area allocation and production of rice in Ethiopia. A total of 4,98,332 tons of rice was produced in the country and out of these 92,562 tons production was produced in Southern Nation and National People of Regional States (SNNPRS) and out of these 90,953 tons production was produced in Kaffa, Benchi Maji and Sheka zones. The rice average yield in Ethiopia is 2.7 tons/ha in upland rain-fed and 3.2 tons/ha in lowland areas (EIAR/ FRG II, 2012).

The major problems in rice production around the world are biotic and abiotic stresses against rice crops (Ou, 1985). One of the biotic stresses in rice crop is blast disease, which caused by a filamentous, ascomycetous fungus, *Pyricularia oryzae Cav*. It is an infectious fungal disease, which is distributed worldwide and prevailing in more than 85 countries of the world ((Jamal *et al.,* 2012) and also as one of the most important disease infecting rice plants in African countries (WARDA, 2004). The disease is a significant problem in temperate regions including Ethiopia and can be found in areas such as irrigated lowland and upland. *Pyricularia oryzae* is otherwise known as rice blast, rice seedling blight, and rotten-neck blast, pitting disease, leaf blast, node blast, panicle blast, collar blast and Johnson's spot (NDPRB, 2011). Blast can be found on the rice plant from the seedling stage to maturity. The leaf blast phase occurs between the seedling and late tillering stages (Couch and Kohn, 2002). Rice blast causes economically significant crop losses annually and causes yield loss as high as 70–80% when pre-disposition factors favor epidemic development (Piotti *et al*., 2005). Several rice blast epidemics have occurred in different parts of the world, resulting in heavy yield losses in these areas ranging from 50 to 90 % of the expected crop (Jamal *et al* ., 2012).

In Ethiopia, blast disease has been recorded on rice in Amhara Region, since 1985 and also in SNNPRS (Abraham, 2008). This disease is one of the major constraints to intensification of rice cultivation. Assessment of the incidence and severity of plant disease is important to determine the disease intensity and status of the disease throughout a studies area in order to prioritize research. Despite the frequent occurrence of severe epidemics of the rice blast disease, there is no detailed information with regards to the rice blast disease intensity against *P. oryzae* pathogen in Ethiopia. *In vitro* condition, these fungi was isolated and identified and also inoculum multiplied on the specific culture media with optimum temperature and hydrogen – ion concentration (pH) (Meena, 2005). But some reports on the effect of culture media, temperature and pH on radial growth as well as on the morphological characters of the pathogen showed contradictory findings. Characterization of *P. oryzae* isolates will be needed in order to determination of cultural (medium), physiological (temperature and pH) and the morphological studies (growth and sporulation) of the native isolates. With this regard it could be vital to conduct the assessment of the rice disease intensity in the South West SNNPRS of Ethiopia and also to identify the domains where rice blast disease may become constraint to the productivity. Therefore, the present study was conducted to determine the intensity of the blast disease and its isolate characterization in upland rice cultivated fields of South west of SNNPRS, Ethiopia.

MATERIALS AND METHODS

Description of the study areas

The assessment of rice blast intensity was carried out in 90 farmers' rice cultivated fields of Kaffa, Bench-Maji and Sheka Zones in SNNPRS South west of Ethiopia during the main cropping season from May to October 2013. The experimental rice research plots at Bonga Agricultural Research Center and one Private Sector Farm from Guraferda district were also included in the assessment.

Table 1 : Study areas environmental conditions.

Zone	District	Altitude range (m)	Longitude (°E)	Latitude (°N)	Temperature °C		Mean annual rainfall(mm)
					min	max	
Kaffa	Gimbo	500 -1950	36° 0′ 0″	7° 15′ 0″	16.7°C	24°C	1710
Benchi-maji	Guraferda	814-1995	34°88' - 36°14'	5°33'-7°.21'	10°C	30°C	1200
Sheka	Yeki	800-1400	35°31'-35°35'	7°24'-7°52'	15.5°C	28.5°C	2298

$$\text{Prevalence (\%)} = \frac{\text{Number of fields affected by the disease}}{\text{Total number of fields assessed}} \text{ X } 100$$

The incidence of rice blast was calculated using the number of infected plants and expressed as a percentage of the total number of plants assessed (Jamal *et al*., 2011).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \text{ X } 100$$

The severity of the disease was examined visually on the whole plants within the quadrants and recorded as the percentage of plant parts (tissue) affected (percentage of blast infection of the plant).

$$\text{Percent severity index (PSI)} = \frac{\text{Sum of numerical rating}}{\text{No. of plants rated * max score of scale}} \text{ X } 100$$

The assessment of disease intensity was conducted at Kaffa zone, Gimbo district (Argoba and Choba localities), Benchi - Maji zone, Guraferda district (Otuwa, Berji and Kuja localities) and at Sheka zone, Yeki district (Alamo locality). A total of 6 localities, two from Kaffa zone, three from Bench-Maji zone, and one from Sheka zone were assessed for rice blast intensity. The description of the study areas altitude, longitude, latitude, temperature and mean annual rainfall is given in table 1:

Assessment of blast disease intensity

The survey was conducted using simple random sampling method, within at 2-3 km intervals on rice fields along the main and accessible road sides. The rice blast incidence and severity were recorded along the two diagonal 'X' fashion of the fields at five random spots using $1m^2$ quadrants and used to calculate the average values. Totally, 90 farmer's rice fields were surveyed at critical growth stage of the crop (tillering) during which the blast symptoms reached its maximum severity level. From each locality, 15 farmer's rice fields were selected. The prevalence of the disease was calculated using the number of fields affected by the disease divided by the total number of fields assessed and expressed in percentage. Scoring scale of blast disease under field condition was rated according to the International Rice Research Institute (IRRI) scale of 1-9 (0 = No lesions; 1 = Small brown speaks of pin point size or large brown speck without speculating centre; 2 = Small round dish to slightly elongated necrotic grey spots about 1-2 mm in diameter with distinct brown margin lesions are mostly found on lower leaves; 3 = Lesion type is same as in scale 2, but significant number of lesion are one on upper leaves; 4 = Typical susceptible blast lesion, 3 mm or longer infecting lesions on 2% of leaf area;5 = Typical blast lesion infecting 2-10% of the leaf area; 6 = Typical blast lesion infecting 11-25% of the leaf area; 7 = Typical blast lesion infecting 26-50% of the leaf area; 8 = Typical blast lesion infecting 51-75% of the leaf area; 9 = More than 75% leaf area affected) (IRRI, 1996; 2009). Data on geographical information (longitude, latitude, and altitude) of each field were recorded using GPS (Trex Legend GPS system, Garmin).

Diseased plant sample collection

Blast infected rice leaf samples at vegetative stage (tillering 3rd critical growth stage) were collected from farmer's fields at an altitude ranges between 1107 and 1423 m. a. s. l. Infected leaves were cut from the mother plant and placed in an envelope, which were labeled with all necessary informations including the name of the region, zone, district, localities, cultivars, GPS data and date of collection. Samples were kept in refrigerator at 4^oC until the surveys in all the districts were finalized. Then after, samples were preserved in ice box and transported to Ambo University Plant Pathology Laboratory for pathogen identification and characterization.

Isolation, purification and identification of rice blast isolates

The oat meal agar (40 g of rolled oats, 5 g of sucrose, and 16 g of agar and 1000 ml of distilled water), and potato dextrose agar media (200g of peeled potatoes, 20 g of dextrose, and 20 g of agar and 1000 ml of distilled water) were used for the isolation of blast pathogen. Diseased leaves of rice cultivars infected with pathogen were cut into small pieces (less than 1.0 cm in size) around the area showing the blast lesion including the edge of the lesion and were surface sterilized with 1% sodium hypochlorite for 1 min. followed by 3 washes with sterile distilled water. Then the plant pieces were placed in Petri dishes lined with moist filter papers and it was incubated at 25 ^0C for 24 h to encouraged sporulation. After incubation, these infected leaf pieces were examined under stereo-dissecting microscope. Abundant pathogen growth and sporulation were observed from in and around the lesions with grey, dense and bushy appearance. A sterile moistened needle was used to pick out some conidia by brushing the needle across the sporulating lesion. The conidia were placed on oat meal agar and potato dextrose agar media plates containing streptomycin (WARDA, 2004).

Plates were incubated at 25°C for about 7-10 days with 12 h darkness and 12 h light. The identity of *P. oryzae* was verified by checking the conidia under light microscope (WARDA 2004). Identification of the pathogen was carried out according to the cultural and morphological characteristics as described by Agrawal *et al*., (1989) and Mew and Gonzales, (2002). The blast isolates designated as *Po* denotes *Pyricularia oryzae* and number denotes representative of sites based on Meena, (2005).

Isolates of the blast pathogen were isolated and purified using single spore (mono conidial) technique (Hoang Dinh *et al.,* 1999). Water agar medium (30 g of agar, 1000 ml of distilled water, 40 mg streptomycin, 5 g of sugar and 2 g of yeast extract) was used for the purpose of single conidial isolation Mono-conidial cultures were isolated from the field blast pathogen isolates, which were derived by streaking a loopful of conidial suspension across a water agar plates in a "W" pattern, thus spreading the conidia. A guideline can be drawn on the undersurface of the plate. Following 24 h incubation at 25^0C, germinating conidia can be easily picked up and sub cultured on to a fresh oat meal agar and potato dextrose agar media plates amended with streptomycin using a fine scalpel.

Characterization of the rice blast isolates

Evaluation of different culture media for growth of P. oryzae isolates

The four culture media viz. oat meal agar media (40 g of rolled oats, 5 g of sucrose, 16 g of agar and 1000 ml of distilled water), rice flour agar media (15 g of complete rice flour, 4 g of yeast extract,15g of agar and 1000 ml of distilled water), malt extract agar media (35.5 g of malt extract agar, and 1000 ml of distilled water) and potato dextrose agar media (200g of peeled potatoes, 20 g of dextrose, 20 g of agar and 1000 ml of distilled water) were used to compare the growth rate of *P. oryzae* isolates after 10 days inoculation (Meena, 2005). From the margin of actively growing of *P. oryzae* isolates; 6 mm diameter mycelia discs of the 14 day old cultures of different *P. oryzae* isolates were inoculated on the middle of the Petri plates and three replications were maintained for each media. The inoculated Petri plates were kept at 30°C. The colony diameter of the growth of each isolate was measured after 10^{th} day of the incubation period and the growth was calculated in mm with the help of a scale. The different colony characters like pigmentation, color of mycelia, surface texture, margin, mycelial growth, sporulation and size, shape and septation of conidia were recorded in all four media by visual and microscopic observations (Meena, 2005). The sporulation capacity of each isolate on different media was assessed by microscopic observations. A loopful of culture was transferred to a clean slide and mixed well with lactophenol and a cover slip was placed on it. The rate of sporulation was recorded in microscopic fields at 40X (Excellent >30; Good- 20-30; Fair-10-20; Poor-<10 and Nil-0) (Meena, 2005). Six *P. oryzae* isolates were identified. All the six isolates of *P. oryzae* were multiplied on OMA for 14 days and spores were collected. The length and width of 10 spores were measured under high power objective (40 X) for each isolate using a micrometer. The average size and shape of conidia (length and width) was determined using ocular and stage micrometer. Number of septa and color were also recorded (Chipili *et al*., 2004).

Effect of temperature on growth of blast isolates

The effect of temperature levels on growth of only selected isolates of *P. oryzae isolates* were grown on potato dextrose agar media. Mycelial discs of the 10 day old culture of *P. oryzae isolates* (6 mm diameter discs) were placed on the middle of PDA Petri plates and incubated at seven different temperatures level i.e., 15, 20, 25, 30, 35, 40 and 45^0C. The experiment was laid out in Completely Randomized Design (CRD) with three replications. After five days of incubation, the colony diameter of each isolate was measured in mm (Getachew *et al.,* 2013).

Effect of pH on the mycelial growth of blast isolates

The growth of the pathogen was also measured in terms of mycelial dry weight. The effect of pH on the growth of the pathogen was studied by the method of Meena, (2005) using potato dextrose broth media. Potato dextrose broth was prepared in 250 ml Erlenmeyer flask, each containing 30 ml broth basal medium. The pH of the broth was adjusted to 3, 3.5, 4, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0.with a help of digital pH meter using 0.1 N HCL and 0.1 N NaOH. Reaction of the medium was adjusted to the desired pH by using di-hydrogen phosphate citric acid buffer according to the method of Vogel (1951). Then the medium was sterilized in autoclave at 120°C for 15 min. From 10 day old culture of *P. oryzae* (6 mm diameter discs) were cut and inoculated into 30 ml basal medium in 250 ml flask and incubated at 30°C. The experiment was laid out in Completely Randomized Design with three replications. After five days of incubation at 30 ^{0}C, the dry weight of the mycelium of each isolate was recorded. After the given incubation period, the mycelial mat of the pathogen was removed and collected in pre-weighed what man's filter paper No 42 and the filter papers with mycelial mat were dried at 60^0 C for 6 days in electric oven. After drying, the filter papers with mycelium were re-weighed. The mycelial dry weight per culture was determined by subtracting the weight of filter paper from the weight of filter paper + mycelial mat (Meena, 2005).

Pathogenicity test for P. oryzae isolates

Once the pathogen was isolated and identified, the pathogenicity test of the pathogen was necessarily for verification, that the fungus was the real cause of the rice blast disease or not. The pathogenicity of all six purified isolates of *P. oryzae* was confirmed by Koch's postulates by the method of Chevalier *et al.,* (1991) under greenhouse conditions at Ambo Plant Protection Research Center Ambo, Ethiopia. The greenhouse soil was prepared from field soil (sandy loam) : sand mixture (3:1), respectively and the soil was autoclaved at 121°C for 2 h. Disinfected viable seeds of Guraferda local rice cultivars were sown in autoclaved sterilized soil using 6 cm diameter pots with 5 plants per pot. The plants were inoculated after germination, at the age of 3-4 leaves and the seedlings in each pot was sprayed with 40–50 ml of spore suspension adjusted to 10^5 spores/ml with the help of haemocytometer. Atomizer sprayer was rinsed with 95% Ethanol and then washed with sterile distilled water and used for spraying (Hans *et al.,* 2003). Inoculated pots were placed on a rotating rack and were sprayed all plants simultaneously while rotating the rack to ensure even coverage of the leaves. The conidial suspensions were sprayed on to the rice seedlings until runoff while water was used for spraying the control treatment. The plants were placed inside the dew chamber at 25°C for 24h and then transferred them to a greenhouse bench at 25-30°C. The severe symptoms were observed on inoculated plants. The symptoms of the lesions that appeared on the leaves of the inoculated plants were observed and compared similar to the symptoms described on the naturally infected plants from the field. Periodical observations were made for the development of symptoms on the leaves starting 7 days after inoculation. Experiments were conducted in CRD with three replications. The fungus was re isolated from the artificially inoculated rice seedlings leaves showing typical *P. oryzae* symptoms and the culture obtained was compared with the original culture and was found to be similar for its morphology and colony characters.

Statistical analysis

The prevalence, incidence and severity data were analysed by using the descriptive statistical analysis (means) over district / localities and altitudinal ranges. Analysis of data on disease parameters were subjected to perform by statistical analysis using SAS software version 9.0 used in Complete Randomized Design with three replications. Also charts using of Excel were drawn and comparison of means were conducted with Duncan's multiple range tests at 5 %

statistical probability level to examine mean statistical differences among treatments.

RESULTS AND DISCUSSION

Assessment of blast disease intensity

During the survey, the rice blast was observed on 100% of all the 90 rice cultivated inspected fields at variable levels (Table 2). The results of the assessment revealed that the intensity (incidence and severity) of the disease vary from slight to high intensity depending on the agro-ecological and cultivars differences. The incidence of rice blast in six different localities varied from 42.01 to 85.69%. The highest mean incidence of rice blast was recorded in Otuwa (85.69%) followed by Berji (75.5 %), while the lowest was recorded in Argoba locality (42.01%).The overall mean incidence for the six localities of three districts of South west of Ethiopia (SNNPRS) was reached 65.68% (Table 2). Likewise, blast severity also showed similar trend as that of incidence in the six localities. The highest severity was recorded in Otuwa locality with range of 8.88 - 88.8 % and mean severity values of 55.7%. This was followed by Berji locality, with a range of 22.22 - 84.4% and mean severity of 55.4% while the lowest severity was recorded in Argoba locality with range of 2.22-73.33% and means severity values of 33.62%. The overall mean severity for the six localities in three districts of the South west SNNPRS reached 47.15% (Table 2). The assessment of blast disease showed that the disease incidence and severity were varied from field to field and locality to locality due to the different geographical and environmental conditions prevailing in each locality. All the assessed six localities were exhibited 100 % blast disease prevalence. This indicated that the rice blast disease was occurred over all areas found in selected localities.

Among the fields inspected, 100 % of the fields assessed were found at altitudes below 1500 m. a. s. l. (lowlands agro ecology). The prevalence of the blast disease was recorded at the lowland elevation agro ecology zone (1107-1423 m. a. s. l.). In a similar way, at the altitude of 1107 - 1290 m. a. s. l., the incidence of the rice blast disease recorded 85.6, 75.5 and 48.4% and severity of the rice blast disease was 55.7, 55.4 and 48.4%. At the altitude of 1327-1423 m. a. s. l., the incidence of the rice blast disease were 66.5, 54.6 and 42.01% and severity of the rice blast disease were 47.7, 42.07 and 33.6%. This result showed that blast severity and incidence was increased with decreased altitude range but lower severity and incidence was observed with increased altitude (Table 3). The higher incidence and severity was recorded in Guraferda district but the lowest incidence and severity was recorded in Gimbo district. WARDA, (2004) reported that the incidence/severity of rice blast varies across different locations and cultivars in different years and blast was prevalent and severe in the rice plants at the vegetative stage. Nutsugah (1997) and Nutsugah and Twumasi (2001) identified the disease as a serious threat to rice production in Ghana. The incidence and severity of blast across rice growing areas in Ghana have been surveyed and areas of low - high blast incidence were identified Nutsugah and Twumasi (2001).

Prevalence, incidence and severity of blast disease across rice cultivars

The assessment of incidence and severity of blast disease revealed that varied depends on cultivars. The most dominant cultivar grown by the farmers in the South west of Ethiopia was Guraferda local but the other cultivars were recently introduced which were grown in few farmers fields, private sector farm and Bonga Agricultural Research Center (Table 4). During the assessment, cultivars such as Guraferda local, Getachew, Hidassie, Tana, Andassa and Suparica-1 were scored highest incidence and severity of blast disease whereas cultivars such as Nerica -3, Nerica -4, Nerica -12 and Kokit were scored lowest incidence and severity (Table 4).

Table 2: Prevalence, incidence and severity of blast disease in rice cultivated fields at six localities of South west Ethiopia.

Blast survey areas			No. of fields inspected	Infected fields	Prevalence (%)	Incidence (%)		Severity (%)	
Zone	District	Localities				Range	mean	Range	mean
B.Maji	Guraferda	Otuwa	15	15	100	41.48-100	85.69	8.88-88.88	55.70
		Berji	15	15	100	34.6-100	75.5	22.22-84.44	55.40
		Kuja	15	15	100	30.56-100	69.8	13.33-84.44	48.44
Sheka	Yeki	Alamo	15	15	100	0.606-100	54.61	2.22-91.11	42.07
Kaffa	Gimbo	Choba	15	15	100	0-100	66.5	0-82.22	47.70
		Argaoba	15	15	100	1.85-100	42.01	2.22-73.33	33.62
Total			90	90					
Mean			15	15	100	18.18-100	65.68	8.28-81.85	47.15

Table 3: Prevalence, incidence and severity of blast disease in rice cultivated fields across altitude range in districts.

Altitude range (m)	No. of fields inspected	Infected fields	Prevalence (%)	Incidence (%)	Severity (%)
1107-1223	15	15	100	85.69	55.70
1163-1277	15	15	100	75.5	55.40
1144-1290	15	15	100	69.8	48.44
1327-1405	15	15	100	54.61	42.07
1367-1389	15	15	100	66.5	47.70
1387-1423	15	15	100	42.01	33.62
Mean	15	15	100	65.68	47.15

Table 4: Prevalence, incidence and severity of blast disease in rice cultivars fields.

Cultivars	Fields inspected	Prevalence (%)	Incidence (%)	Severity (%)
Kokit	1	100	24.6	3.33
Hidassie	1	100	91	78.34
Suparica-1	1	100	83.3	73.4
Nerica-3	3	100	20.7	2.6
Nerica-4	12	100	23	2.6
Andassa	1	100	87.6	60.2
Tana	1	100	92.2	75.4
Getachew	1	100	94.6	80.2
fofi3737	1	100	74	47.03
fofi3730	1	100	81.6	54.2
Guraferda local	76	100	96.7	86.3
Nerica 12	1	100	18.9	2.2
Mean			65.68	47.15

CHARACTERIZATION OF THE RICE BLAST ISOLATES

Evaluation of different culture media on sporulation of blast isolates

Sporulation of each *P. oryzae* isolate on 14th day of different media showed significant differences between isolates. Totally six *Po* isolates (*Po*12, *Po* 28, *Po* 41, *Po* 55, *Po* 72 and *Po* 85) were identified from 90 blast samples based on growth characteristics of the test pathogen and geographical location of the origin of the isolate. All the six *Po* isolates were observed good sporulation on media of oat meal agar (OMA) and also good sporulation was observed on rice flour agar (RFA) for the following four isolates *Po* 12, *Po* 28, *Po* 41, and *Po* 85. The fair sporulation was observed on RFA media for the isolates of *Po* 55, and *Po* 72 and on potato dextrose agar (PDA) media for the isolates of *Po* 41, *Po* 72 and *Po* 85. The poor sporulation was recorded on PDA media on isolates of *Po* 12 and *Po* 55 and malt extract agar (MEA) media on isolates of *Po* 28, *Po* 41, *Po* 55, *Po* 72 and *Po* 85, respectively. Similar results were also reported by Afshana *et al*., (2011) and Bandyopadhyay *et al*., (2009) found that the OMA media was suitable and good for sporulation of blast isolates. Also, Gopal *et al*., (2012) reported that among the different media used, OMA media was found to be the best for sporulation of the blast isolates from both rice and finger millet. In the present study also among the four medias, OMA media was the good sporulation of all the six *Po* isolates viz., *Po* 12, *Po* 28, *Po* 41, *Po* 55, *Po* 72 and *Po* 85.

Colony growth diameter of rice blast isolates on different culture media

The results revealed that there is a considerable variation among the colony diameter of the *P. oryzae* isolates on different solid medias (Table 5). The mean of radial growth of different isolates on different solid media, the OMA and RFA wee optimum for all the cultures of *Po* isolates. The radial growth of five *Po* isolates viz., *Po* 12 (88 mm), *Po* 72 (85.3 mm), *Po 55* (83.6 mm), *Po* 28(83.3 mm) and *Po* 41(82mm) on the 10thday showed significantly highest on OMA media followed by isolate *Po* 85 (80.3 mm). On RFA media, the highest colony growth diameter was recorded significantly in *Po* 12 isolate (86 mm) followed by the isolates of *Po* 28 (77.6 mm), *Po* 41(77 mm), *Po* 72 (76 mm) and *Po* 55 (75.6mm), whereas the *Po* 85 (55.6mm) isolate showed the least radial growth. On PDA media, the highest colony growth diameter was recorded significantly only in *Po* 85 (75.6 mm) isolate followed by the isolate of *Po* 28 (61.3 mm) whereas *Po* 55 isolate showed the least radial growth (55.3 mm). On MEA media, the highest colony growth diameter was observed significantly on isolates of *Po* 85 (59 mm) and *Po* 28 (53.3 mm) followed by the isolates of *Po* 41 (50mm) and *Po* 72 (49.6 mm) while the least growth was observed on isolates of *Po* 12 (44 mm) and *Po* 55 (44 mm). Among the four media, the maximum radial growth of the isolate was observed in OMA (88 mm) of isolate *Po* 12 followed by RFA (86 mm) of isolate *Po* 12 and the least radial growth was observed in MEA (59 mm**)** of isolate *Po* 85. Similarly, Kulkarni (1973) reported that among the solid and liquid medias, OMA was found to be good for growth of the *P. oryzae* isolates. Similar results were also reported by Afshana *et al*., (2011**)** that the OMA was suitable for growth of *P. oryzae*. The present study results were also supported by Gopal *et al*., (2012) among the different media used, OMA was found to be the best for mycelial growth of the isolates of *P. oryzae* from rice and finger millet crop plants.

Colony characteristics of Po isolates on the different culture media

Significant variation among the colony characteristics of the different isolates were observed in culture plates which were collected from different localities. All the six *Po* isolates grown on four different media were observed the mycelial color, margin, pigmentation, surface texture and growth. On the OMA medium, the isolate *Po* 12 colony color was dark gray and the light gray color in the isolate *Po* 85 and off white color in isolates *Po* 12 and *Po* 72 and grayish

Table 5 Colony diameter of *Po* isolates *on* different solid medias

Isolate	Mean Colony diameter(mm) 10 DAI				Mean
	Media				
	PDA	**OMA**	**RFA**	**MEA**	
Po 12	52 E	88 A	86 A	44 C	67.5AB
Po 28	61.3B	83.3AB	77.6B	53.3 AB	68.87A
Po 41	53.6D	82 AB	77 B	50 B C	65.65B
Po 55	47.6F	83.6AB	75.6B	44 C	62.7C
Po 72	55.3C	85.3AB	76 B	49.6 BC	66.55AB
Po 85	75.6A	80.3B	55.6C	59A	67.62AB
Mean	57.56C	83.75A	74.63B	49.98D	66.48
LSD(0.05)	0.93	6.90	7.95	6.87	
CV(%)	0.91	4.63	5.98	7.73	

Means followed by the same letters are not significantly different at the 5 % level by DMRT.

Table 6 Colony characteristics of *Po* isolates on different solid media

Isolates	**Media**	**Colony characters**					
		Colony color	Margin	Pigmentation	Surface texture	Mycelial growth*	Sporulation
Po 12	PDA	Grey	Irregular	black`	cottony	Medium growth	Poor
	OMA	Dark gray	Entire	black	Velvety	Good growth	Good
	RFA	Light Grey	Entire	black	Velvety, thick	Good growth	Good
	MEA	olive gray	Irregular	Dark brown	cottony	Poor growth	Poor
Po 28	PDA	Grey	Entire	black`	cottony	Medium growth	Fair
	OMA	off white	Entire	black	Velvety	Good growth	Good
	RFA	Light Grey	Entire	black	Velvety, thick	Good growth	Good
	MEA	olive gray	Irregular	Dark brown	cottony	Medium growth	Poor
Po 41	PDA	Grey	Entire	black`	cottony	Medium growth	Fair
	OMA	Grayish Black	Entire	Black	Velvety	Good growth	Good
	RFA	Light Grey	Entire	Black	Velvety, thick	Good growth	Good
	MEA	olive gray	Irregular	Dark brown	Velvety, thick	Medium growth	Poor
Po 55	PDA	Grey	Irregular	black`	cottony	Poor growth	Poor
	OMA	Grayish Black	Entire	Black	Velvety	Good growth	Good
	RFA	Light Grey	Entire	Black	Velvety, thick	Good growth	Fair
	MEA	olive gray	Irregular	Dark brown	cottony	Poor growth	Poor
Po 72	PDA	Light greyish	Irregular	black`	cottony	Medium growth	Fair
	OMA	off white	Entire	Black	Velvety	Good growth	Good
	RFA	Light Grey	Entire	Black	Velvety, thick	Good growth	Fair
	MEA	olive gray	Irregular	Dark brown	Velvety, thick	Poor growth	Poor
Po 85	PDA	Grey	Entire	black`	cottony	Good growth	Fair
	OMA	Grayish Black	Entire	Black	Velvety	Good growth	Good
	RFA	Light Grey	Entire	Black	Velvety, thick	Medium growth	Good
	MEA	olive gray	Irregular	Dark brown	Velvety, thick	Medium growth	Poor

*Growth (mean colony diameter)

Good = 75-90 mm

Moderate = 56-75 mm

Low/poor = <56 mm (Narendra, 2006).

black color in the other three isolates, *Po* 41, *Po* 55 and *Po* 85 were noticed. Colony of all the isolates margins were entire and the pigmentation was black, velvety in surface texture and good in growth. On the RFA medium, the colony of all the isolates were light gray in color, entire in margin, black in pigmentation, velvety and thick in surface texture and good in growth. On the MEA media, the colony of all the isolates were olive gray in color, irregular in margin, and dark brown in pigmentation. The isolates of *Po* 12, *Po* 28 and *Po* 55 were cottony surface texture whereas the other three isolates (*Po* 41, *Po* 72 and *Po* 85) were velvety and thick in surface texture. The colony of isolates, *Po* 28, *Po* 41 and *Po* 55 were medium in growth whereas the other isolates (*Po* 12, *Po* 55 and *Po* 72) were poor in growth. On the PDA media, the colony of the five isolates (*Po* 12, *Po* 28, *Po* 41, *Po* 55 and *Po* 85) wee gray in color whereas the isolate *Po* 85 was light gray. The colony margin of the isolates of *Po* 12, *Po* 55 and *Po* 72 were irregular while the other three isolates, *Po*28, *Po*41 and *Po*85 margins were regular. All the isolates pigmentations were observed black in color and the surface texture were noticed cottony. The isolate, *Po*85 was observed good growth and the isolates of *Po* 12, *Po* 28, *Po* 41, *Po* 55 and *Po* 72 were medium in growth whereas the isolate *Po*72 was poor in growth (Table 6).

Similarly, Meena (2005) also reported that the colony characteristics of *Po* isolates on OMA media showed greyish black and entire colony margin and also showed both irregular and entire colony margins of some isolates on PDA medium. Bandyopadhyay, (2009) was reported that the OMA media produced off-white, good and regular mycelial growth. These results also agreed with Gopal *et al*, (2012) in rice blast isolates of the color of colony showed grey and black grey for the finger millet isolates on OMA medium. The present study results were also supported by Arunkumar and Singh (1995) that the *Po* isolates showed best performance in OMA for the rate of colony growth. Agarwal *et al*., (1989) and Vanaraj, (2013) stated that the colony color of *Po* isolates were appeared grey on PDA medium. Mew and Misra (1994) reported that the colonies of *Po* isolates on PDA medium showed blackish in pigmentation. Also in this present study, the pigmentation color was observed black. Mew and Gonzales, (*2002)* indicated that the *P. oryzae* pathogen colonies on PDA medium grow very slowly and colony on the reverse side of the agar plates were black.

Conidial characteristics of rice Po isolates

The morphological characteristics of six different isolates of *Po* on OMA only were observed. The results showed that all of the conidia in each isolate was pyriform in shape, base rounded, apex narrowed, two-septate, with three celled observed in isolates of *Po* 28, *Po* 55, *Po* 72 and *Po* 85 and one-septate with two celled was observed in isolates of *Po* 12 and *Po* 41. The conidium in each isolate was observed hyaline to pale olive colors. Among the different isolates, the morphological variability in respect to conidia length and width were also observed significantly. The observed conidial length was varied from 14.5-26.5µm. The maximum length of the conidia was recorded in isolates of *Po* 55 (24.73 µm), *Po* 12 (23.5 µm) and *Po* 41 (21.66 µm) followed by isolates of *Po* 28 (19.96 µm) and *Po* 72 (18.93µm) and shortest conidia was observed in *Po* 85 isolate (18.6µm). The observed conidial width was varied from 5.1-8.3 µm. The maximum width of the conidia was observed on the *Po* isolates of *Po* 28 with (7.86 µm) and *Po* 72 (7.59 µm) followed by *Po* 12 (6.56µm) whereas the narrowest width was observed in isolates of *Po* 85 (6.04 µm), *Po* 41 (6.03µm) and *Po* 55 (5.96 µm), respectively (Table 7). Ono and Nakazato, (1958) observed that the size of conidia of *P. oryzae* varied with the culture media. The sizes as well as shape of the spores and colonies of filamentous fungi are the most important factors in fungal identification. The present study results were also supported by the other workers (Mew and Gonzales 2002; Meena 2005 and Afshana *et al.,* 2011).

Table 7: Conidial characteristics of *Po* isolates

Isolate	Conidial characteristics						
	Conidial Size (in µm)				Conidial shape	Conidial color	Septation of conidia
	Length		Width				
	Range	Mean	Range	Mean			
Po 12	21.3-25.1	23.5AB	6.2-7.01	6.56BC	Pyriform	hyaline to pale olive	1-septate ,2 celled
Po 28	17.6-22	19.96BC	7.5-8.3	7.86A	Pyriform	hyaline to pale olive	2-septate,3 celled
Po 41	19.4-23.1	21.66ABC	5.5-7	6.03C	Pyriform	hyaline to pale olive	1-septate ,2 celled
Po 55	22.3-26.5	24.73A	5.1-6.7	5.96C	Pyriform	hyaline to pale olive	2-septate,3 celled
Po 72	16.7-21.3	18.93BC	7.2-8.25	7.59AB	Pyriform	hyaline to pale olive	2-septate,3 celled
Po 85	14.5-21.01	18.60C	5.8-6.5	6.04C	Pyriform	hyaline to pale olive	2-septate, 3 celled
Grand mean		21.23		6.67			
LSD (0.0)%		4.33		1.07			
CV (%)		11.46		9.02			

Table 8:Effect of different temperatures on the mycelial growth of *Po* isolates grown on PDA media

Isolate No.	Mean Colony diameter (mm) at different temperature levels in(^{o}C)							
	15	20	25	30	35	40	45	Mean
Po12	13.33B	27.66AB	44.66C	56ABC	30.66A	5.66B	NG	25.424C
Po 28	16.66AB	27.66AB	38.66D	52C	30.66A	7AB	NG	24.663C
Po 41	18.33A	25.33ABC	54.33A	57.66AB	32.66A	8.66A	NG	28.139A
Po 55	14.33AB	28A	44C	60.33A	32.66A	7AB	NG	26.617B
Po 72	13.66B	22C	50B	55.33BC	30A	6B	NG	25.284C
Po 85	12.66B	24BC	41.66CD	59.33AB	32.66A	6.66B	NG	25.281C
Mean	14.83E	25.77D	45.55B	56.77A	31.55C	6.83F	G	
LSD (0.05%)	3.97	3.48	3.35	4.31	3.88	1.77		
CV (%)	15.07	7.59	4.13	4.27	6.92	14.63		

In a column, means followed by a same letters are not significantly different at the 5 % level by DMRT.

Effect of temperature on growth of *Po* isolates

Temperature is one of the most important physical environmental factors for regulating the growth and reproduction of *P. oryzae* isolates. The effects of different temperatures on the mycelial growth of six *Po* isolates were studied. The results showed that the colony diameter of each *Po* isolates on the 5th day after inoculation on different temperature levels showed significantly differences between different temperatures and isolates. Among the six different isolates, the radial growth of isolates of *Po* 55 (60.33 mm), *Po* 85 (59.33 mm), *Po* 41(57.66 mm) and *Po* 12 (56 mm) on the 5th day after inoculation were significantly highest on the temperature level of 30^{o}C followed by the *Po*72 isolate with 55.33 mm whereas the minimum growth was recorded at *Po* 28 isolate with 52mm (Table 8).

At the temperature level of 25^{o}C, among the six different isolates, *Po* 41 isolate colony radial growth on the 5th DAI was significantly highest (54.33 mm) over the other isolates followed by the *Po* 72 isolate (50mm), whereas the minimum growth was recorded in isolate of *Po* 28 (38.66 mm) (Table 8). On the temperature level of 35^{o}C, the radial growths of all isolates were not observed significantly difference between them. Minimum growth was observed in temperature level of 40^{o}C and 45^{o}C. At the temperature of 40^{o}C the different isolates of radial growth, *Po* 41

Table 9: Effect of different pH levels on the mycelial growth of *Po* isolates grown on PD broth media.

Isolates	Dry mycelium weight (mg) at different pH level									
	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	Mean
Po **12**	6.66A	11ABC	19.33AB	51.66AB	84A	115.33AB	249C	323.33A	206.66B	89.66B	32.66B	108.11B
Po **28**	4.33AB	7.33C	22.66AB	53A	74C	92C	265.33B	304.66B	184.33C	93.33A	28.66B	102.69C
Po 41	6AB	8.66BC	21AB	39.66C	65D	109B	293.33A	281.33C	171D	94A	33.66AB	102.05C
Po 55	4.76AB	13AB	24.33A	47.33C	60F	125.33A	229.33D	309.66B	180C	95.66A	31.33B	101.20C
Po **72**	3.6B	14A	17C	41.66C	63E	100.66BC	284A	318.33A	164.66E	85C	32.33A	102.20C
Po **85**	6.66A	11.66ABC	22AB	54A	80B	115.66AB	293.66A	318A	216.66A	85.66C	38A	112.90A
Mean	5.33K	10.94J	21.05I	47.88G	71F	109.66D	269.11B	309.22A	187.22C	90.55E	32.77H	
LSD	2.35	4.10	3.16	4.89	0	14.85	12.6	7.5	6.26	2.93	5.06	
CV	24.7	21.1	8.45	5.7	0	7.61	2.63	1.36	1.88	1.82	8.68	

In a row, means followed by the same letters are not significantly different at the 5 % level by DMRT.

isolate was significantly highest (8.66 mm) over the other isolates followed by the isolates of *Po*28 (7), *Po*55 (7), *Po* 85 (6.66), *Po* 72 (6.66) and *Po* 12 (5.66 mm) but all the six isolates were not grown on the temperature level at 45°C. The ideal temperature range for the maximum growth of different isolates of *P. oryzae* was observed from 25°C to 30°C temperature levels followed by temperature levels of 35°C and 20°C and the minimum mycelial growth was recorded at the temperature levels of 15°C, 40°C and 45°C. The mycelial growths of different *P.oryzae* isolates were highly suppressed at temperatures of 15°C, 40°C and 45°C. It was observed that after 20°C, the colony growth was increased and attained maximum at 30°C and then start reduced **(**Table 8). Similarly, Jamal *et al*., (2012) and Bahadur *et al.,* (2013**)** also reported that *P. oryzae* isolates grew at 30°C was the optimum temperature for mycelial growth. The present study results were also supported by Meena, (2005) at the temperature of 30°C was the optimum temperature for the mycelia growth of the *Po* isolates. In this study, the growth of all the isolates was decreased drastically at 40°C. Arunkumar and Singh (1995) studied the differential response of *P. oryzae* isolates from rice, finger millet and pearl millet related with temperature and they reported that all the isolates exhibited maximum growth at 30°C. The present study results are also in close agreement with those already reported by Hossain *et al*., (2004) who reported 30°C as optimum temperature for growth of *P. oryzae*.

Effect of pH on the mycelial growth of Po isolates grown on PD broth media

The results revealed that there was a considerable variation among the dry mycelial weight of the different isolates on different pH concentrations. The maximum mean dry mycelial weight was observed at pH 6.5 (309.22 mg) followed by pH 6 (269.11 mg) and pH 7 (187.22 mg). But the least mean dry mycelia weight was recorded at pH 3 (5.33mg), pH 3.5 (10.94mg), pH 4 (21.05), and pH 8 (32.77mg).

The dry mycelial weight at pH 6.5 (323.3-318mg) for isolates of *Po*12, *Po* 85 and *Po* 72 were significantly difference over the mycelial weight for other isolates. The dry mycelial weight (293.7-284mg) at pH 6 for the isolates of *Po* 85, *Po* 41 and *Po* 72 were significantly difference over the mycelial weight for other isolates (Table 9). The dry mycelial weights of *Po* isolates were significantly increased from pH 3.0 to pH 6.5 and which further start too deteriorated. The results of the present study indicated that *Po* isolates prefers pH range of 6.00- 6.50. The pH below six and above seven was observed to be hindering for the growth of *Po* isolates. Therefore, at pH 6.5 almost all *Po* isolates revealed maximum dry mycelial weight; this showed that it is an optimum pH for growth of *Po* isolates (Table 9). The present study results were also supported by Arunkumar and Singh (1995) who obtained the best growth of the *Po* isolates at pH 6.5 and Mijan Hossain (2000) reported that growth of *Po* isolates increased with increase in pH from 3.5 to 6.5. The present study results were strongly supported by Meena (2005) who reported that the growth of all the isolates at pH 6.5 was significantly superior, over other treatments and growth of all the isolates significantly increased from pH 3.0 to pH 6.5 which further started declining and the least growth was observed at pH 8.0.

Pathogenicity test for Po isolates

Pathogenicity test results revealed that the disease symptoms and development of six *Po* isolates on susceptible local cultivar after inoculation with the inoculum of the test *P. oryzae* isolates. The diamond and spindle shaped with gray center and dark brown to necrotic margins were observed on all of the rice seedlings after the 7th day of inoculation. After 7 day of inoculation, 86.66% disease incidence with 63.2 % of average disease severity was recorded in *P. oryzae* inoculated rice plants, whereas the disease was not developed in un inoculated rice plants. The *P. oryzae* isolates were re-isolated from the infected lesions and compared with the original culture and thus Koch's postulates was proved. The re-isolation revealed that the isolated fungi from diseased rice seedlings were found to be identical with those used for artificial inoculation. Although the reaction types showed by local susceptible rice cultivar to *P. oryzae* isolates were similar in both field and greenhouse but the disease severity was more intense in the field of the selected localities. All the isolates were the causative agents for blast disease of rice. Pathogenicity test revealed that all *P. oryzae isolates* were able to infect local susceptible rice.

CONCLUSIONS

The primary aim of this study was to assess the rice blast disease prevalence, incidence and severity on upland rice cultivars in the South west of Ethiopia and to characterize the rice blast pathogen isolates collected from various South West areas of Ethiopia. It was observed in all assessed localities at variable levels. The results of the assessment revealed that the incidence and severity of the blast disease varied from slight to high intensity from field to field and localities to localities depending on the agro-ecological and environmental conditions prevailing in each locality. The different culture media was evaluated based on colony diameter to get suitable media for the growth of the *Po* isolates. The different media was evaluated on their potential of sporulation of *Po* isolates and good sporulation was observed on the medium of oat meal agar for all the six isolates (*Po*12. *Po*28, *Po*41, *Po*55, *Po* 72 and *Po* 85). The ideal temperatures for the maximum growth of six Po isolates were ranged from 25°C to 30°C temperature levels. The dry mycelial weight of *Po* isolates was significantly increased from pH 3.0 to pH 6.5 and which further start too deteriorated. It could be concluded that rice blast was the most important disease of rice cultivars in South West (SNNPRS) of Ethiopia. Suitable media, optimum temperature and pH level preferred to the mycelial growth and sporulation of the *P. oryzae* isolates and maximum mycelial

growth and sporulation was found in oat meal agar medium followed by rice flour agar medium. The best mycelial growth was also found at temperature 30°C and pH 6.5 which was suitable for mycelial growth of *P. oryzae* isolates. Further studies have to be conducted to evaluate their resistance under green house and natural environmental conditions. To privilege rice production in South West (SNNPRS) of Ethiopia, these diseases that occur concurrently on rice could be manage using the resistant cultivars. Farmers and development agents should be trained in the management of rice blast disease by using resistant cultivars.

REFERENCES

Abdu S, Nonglak P, Prapa Sripichitt and Sreewongchai T (2013) Identification of blast-resistant varieties from landrace, improved and wild species of rice. *Thailand. Kasetsart J. (Nat. Sci.)* 47: 1 – 7.

Abraham T (2008) Increasing crop production through improved plant protection - volume 1.proceedings of the 14th annual conference of the protection society of Ethiopia (PPSE), MOARD19-22 December 2006.*Addis Ababa, Ethiopia*. pp 377.

Afshana BD, Shahijahan M, Hussain DS and Snober HB (2011) Morphological variability among various isolates of *Magnaporthe grisea* collected from paddy growing areas of Kashmir. 8(1): 45-56.

Agrawal PC, Mortensen CN and Mathur B (1989) Seed borne diseases and seed health testing of rice. *Technical Bulletin* No.3, Phytopathological paper No. 30, CAB Int. Mycological Ins. (CMI) Kew, Surrey, UK. pp7.

Arunkumar S and Singh RA (1995) Differential response of *Pyricularia grisea* isolates from rice, finger millet and pearl millet to media, temperature, pH and light. *Indian Journal of Mycology and Plant pathology*, 25:238-242.

Bahadur R, Tiwari KS, Netam RS and Tiwari U (2013) Effect of different culture media, carbon source, nitrogen source, temperature and *p*H, Level on the Growth and Sporulation of *Pyricularia grisea* isolate from finger millet. *Research Journal of Agricultural Sciences*, 4(1): 83-86.

Bandyopadhyay J, Kumar RK, Srivastava L and Bhatt RP (2009) Effect of media on growth, sporulation, and production of perithecia of blast pathogen, *Pyricularia grisea.* Department of Genetics and Plant Breeding, India. 2(1): 37-40.

Chevalier M, Yespinasse Y and Renautin S (1991) A microscopic study of the different classes of symptoms coded by the Vf gene in apple for resistance scab (*Venturia inaequalis*), *Plant Pathology*, 40:249-256.

Chipili JK, Twumasi K, Dartey SK, Nutsugah S, Screenivasprasad Y, Sere Y and Dogbe W (2004) Survey of rice blast and varietal screening in Ghana. West Africa Rice Development Association, 01 BP 2551, Bouaké, Côte d.Ivoire. pp 49-60.

Couch BC and Kohn LM (2002) A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae*, from *M. grisea*. *Mycologia*, 94, 683-693.

EIAR/ FRG II (2012) Backing Rice Extension Rightly. FRG II Project Empowering Farmers' Innovation Series No. 4. Ethiopian Institute of Agricultural Research EIAR-JICA, Addis Ababa, Ethiopia . pp 1-5.

FAO (2012) FAO Rice Market Monitor, Trade and Markets Division Food and Agriculture Organization of the United Nations Volume XV - Issue No. 3: Available online at http://www.fao.org/economic/est/publications/rice-publications/rice-market-monitor.

Getachew G, Tesfaye Alemu and Kassahun Tesfaye (2013) First Bio-Innovate Regional Scientific Conference United Nations Conference Centre (UNCC-ECA) Addis Ababa, Ethiopia, 25-27.

Gopal B, Hira KC, Manandhar K, Ram B, Khadka L, Sundar M and Shrestha L (2012) Study on differential response of *Pyricularia grisea* isolates from rice, finger millet and *Panicum* spp. with Local and Alien Media, and their host range. *Nepal Journal of Science and Technology*, 13: 7-14.

Hans Y, Bonos SA, Clarke BB and Meyer WA (2003) Inoculation techniques for selection of gray leaf spot resistance in perennial ryegrass. *USGA Turfgrass and Environmental Research online*, 2:1-9.

Hoang Dinh, Takahito Noda and Pham Van du (1999) Deployment of resistant varieties to blast (*Pyricularia grisea)* in the Mekong Delta. *Omon RICE*, 7: pp 133-134.

Hossain MM, Srikant K and Hegde YR (2004) Physiological and nutritional studies on *Pyricularia grisea*, the causal agent of blast of rice. Department of Plant Pathology, University of Agricultural Sciences, Dharwad India. *Karnataka Journal of Agricultural Sciences*, 17(4): 851-853.

International Rice Research Institute (IRRI) (1996) Standard evaluation system for rice. 4th.ed. IRRI, Manila, Phillipine.

International Rice Research Institute (IRRI) (2009) Rice Policy-World Rice Statistics (WRS). Retrieved May 28, 2010 from. http://www.irri.org/science/ricestat.

Jamal-u-ddin Hajano G, Mubeen A, Lodhi A, Mumtaz A, Pathan L and Qayoom AR (2011) Rice blast-mycoflora, symptomatology and pathogenicity. Sindh Agriculture University Tandojam, 5 (1): 53-63.

Jamal-u-ddin Hajano G, Serwar Shah M, Ali Khanzada, Mubeen Lodhi and Mumtaz Pathan (2012) *In-vitro* evaluation of fungicides, plant extracts and bio-control agents against rice blast pathogen, *Magnaporthe oryzae* couch. Sindh Agriculture University, Tandojam, Sindh, Pakistan. *Pak. J. Bot*., 44(5): 1775-1778.

Kulkarni S (1973) Studies on the blast disease of *Eleusine coracana* (L.) Gaertn.(Finger millet or Ragi) in Mysore State. M.Sc. (Agri.) Thesis,U.A.S Bangalore. pp104.

Meena BS (2005) Morphological and molecular variability of rice blast pathogen, *Pyricularia grisea* (Cooke) Sacc.Department of Plant Pathology, College of Agriculture, Dharwad University of Agricultural Sciences, Dharwad. Pp 12-54.

Mew TW and Gonzales PA (2002) Handbook of Rice Seed borne Fungi. International Rice Research Institute, Los Banos, Philippines, pp. 83.

Mew TW and Misra JK (1994) A Manual of Rice Seed Health Testing. International Rice Research Institute: Manila, Philippines. pp 83.

Mijan Hossain MD (2000) Studies on blast disease of rice caused by *Pyricularia grisea* (cooke) Sacc. in upland areas. *M.Sc. Thesis,* University of Agricultural Sciences, Dharwad, pp. 53.

MoRAD (Ministry of Agriculture and Rural Development) (2010) National Rice Research and Development Strategy of Ethiopia. Addis Ababa, Ethiopia.

NDPRB (2011) National diagnostic protocol for rice blast on rice caused by *Magnaporthe oryzae* Australian Government Department of Agriculture, Fisheries and Forestry Protocol number 14, 1: 3-7.

Nutsugah SK (1997) Survey of diseases/pests of upland/ hydromorphic rice in Northern Ghana. Proceedings of the SARI Annual In-house Review, March 24.26, 1997, Nyankpala, Ghana.

Nutsugah SK and Twumasi JK (2001) Fungal Diseases of Rice in Ghana: Knowledge and Research Needs. *In*: S. Sreenivasaprasad and R. Johnson, (eds.). Major Fungal Diseases of Rice Recent Advances. Kluwer Academic Publishers. *The Netherlands*, pp. 321.329.

Ono K and Nakazato K (1958) Morphology of the conidia of *Pyricularia* from different host plants produced under different conditions. *Annals of the Phytopathological Society of Japan*, 23: 1 – 2.

Ou SH (1985) Rice Diseases. CAB International Mycological, Institute Kew, Survey, UK.

Piotti E, Rigano MM, Rodino D, Rodolfi M, Castiglione S, Picco AM and Sala F (2005) Genetic structure of *Pyricularia grisea* (Cooke) Sacc. isolates from Italian paddy fields. *Journal of Phytopathology*, 153: 80–86.

Priya Vanaraj, Ramalingam L, Robin Sabariyappan, Saveetha Kandasamy, Sankaralingam Ambalavanan and Rabindran S (2013) Variability in *Pyricularia oryzae* from different rice growing regions of Tamil Nadu, India. *African Journal of Microbiology Research*. 7(26): 3379-3388.

Traoe O, Pinel A, Hébrard E, Dieudomme-Gumedzoe MY, Fargette D, Traore, A and Kanaté G (2006) Occurrence of Resistance –Breaking Isolates of Rice Yellow mottle virus in West and Central Africa. *Plant Dis*., 90: 259-263.

Vogel AI (1951) A Text Book in Organic Quantitative Analysis, Congmcans. Green and Co., London, N. Y., Toronto, pp. 865 – 872.

West Africa Rice Development Association (WARDA) (2004) Rice blast in West Africa: Characterization of pathogen diversity, key screening sites and host resistance. Proceedings of a stakeholder workshop, Project R7552, UK Department for International Development and Crop Protection Programme, *Proceedings Series* no. 3, Vol. 4: pp 123.