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RESEARCH ARTICLE

EVALUATION OF RICE GENOTYPES FOR RESISTANCE AGAINST BROWN SPOT DISEASE CAUSED BY *Bipolaris oryzae*

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ABSTRACT

Brown spot caused by *Bipolaris oryzae* is one of the destructive diseases of rice in the world and causes severe losses in grain quality and quantity. Use of resistant varieties is the sustainable and eco-friendly way of controlling brown spot disease in rice. In this concern, we evaluated 20 rice genotypes under glasshouse and field conditions during June, 2014-May, 2015 to determine the level of resistance against *B. oryzae*. Disease assessment was done by calculating disease severity, area under disease progress curve (AUDPC) and AUDPC per day (AUDPC/day). Response of rice genotypes to brown spot disease was found similar under both the field and glasshouse conditions. The tested genotypes showed variable response to the level of resistance. Mean AUDPC value varied from 80.36 to 340.05. Sabitri appeared the most resistant with AUDPC value of (80.36) followed by Radha-4 (108.02), while Sankharika (340.05) was the most susceptible among the tested genotypes. Grain yield ranged from 2.76 to 4.76 t ha^{-1}. Of the tested genotypes, Sabitri and Sankharika yielded the highest (4.76 t ha^{-1}) and the lowest (2.76 t ha^{-1}) grain yields, respectively. Grain yield was negatively correlated with AUDPC (r = -0.628**). Thus, the genotypes Sabitri and Radha-4 could be utilized as a source of resistance for breeding of rice for brown spot resistance.

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INTRODUCTION

Productivity of rice is very low in Nepal compared to potential and attainable yield and yield obtained in neighboring countries. Among the fungal diseases of rice, brown spot disease which is caused by *Bipolaris oryzae* Subr. and Jain (synonyms *Dreschslera oryzae, Helminthosporium oryzae*) has become the emerging and major threat for yield decline. Brown spot has been widely reported in south-east Asian countries (Reddy *et al*., 2010) and is associated with two major epidemics in India. On an average, the disease causes 10% yield loss across all lowland rice production in South and Southeast Asia and severely infected field can have as high as 45 per cent yield loss (IRRI, 2012). In Nepal, brown spot is classified as a major fungal disease of rice next to blast (Manandhar *et al*., 1987). The pathogen infects coleoptiles, leaves, leaf sheath, panicle branches, glumes, and spikelet (Mew and Gonzales, 2002). Screening of rice varieties against brown spot disease at Jyotinagar, Chitwan district and Paklihawa, Rupandehi district of Nepal showed that many of the rice genotypes were susceptible to brown spot disease (Magar, 2015; Aryal *et al*., 2016). Some tolerant and potential genotypes specific to the location have been also recommended by them. The use of resistant varieties is the most economic and environmental friendly method for the management of the disease (Haq *et al*., 2002) but the resistance is very scarce and not stable due to the appearance of new/more virulent races of pathogens (Katasntones *et al*., 2007). Therefore, resistance level for each rice variety has to be updated each year. In our study, some popular varieties of rice among farmers of Dhanusha district and some promising lines of rice were selected to find out the resistant genotypes against brown spot disease of rice. Cultivation of the resistant genotypes and using them as the source of resistance can be an eco-friendly and sustainable management strategy of brown spot disease of rice.

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MATERIALS AND METHODS

Collection of seeds: Seeds of 20 rice genotypes including some popular varieties among farmers of Dhanusha district and some promising lines were collected from National Rice Research Program (NRRP). Sambha Mahsuri was taken as a susceptible check and Sabitri as a resistant check.

Experimental site: The field experiment was conducted at NRRP, Hardinath, Dhanusha, Nepal which is located at 26º 48' 9.6" N latitude and 85º 57' 37.4" E longitude with an elevation of 73 m from sea level at the eastern terai of Nepal. The area is sub-tropical with humid climate. The maximum and minimum temperature recorded throughout the experiment was 34.98ºC in August and 15.72ºC in November with relative humidity ranging from 73.93% to 96.20%. Maximum rainfall was recorded 331.8 mm in August. The soil of the experimental plot was clay loam having pH 6.3, organic carbon 1.7%, total nitrogen 0.11%, available phosphorus 40 kg/ha and potassium 115 kg/ha.

Experimental design, sowing and transplanting: Glass-house experiment was conducted at the Agriculture and Forestry University (AFU), Rampur in a completely randomized design with four replications. The nursery and field experiments were carried out in a randomized complete block design with three replications at NRRP, Hardinath, Dhanusha during summer season (June to January, 2014/15) under rainfed conditions. The nursery plots were raised 15 cm above the ground. Seeding was done on June 24 @ 40 kg ha^{-1}. Each genotype was seeded continuously in rows of 50 cm length with 20 cm distance between rows was maintained. Each genotype had 10 rows. The individual plot size was 1 m^2. In field, transplanting was done on July 30. Twenty-one day old seedlings were transplanted on perpendicular rows with the length of plot at the distance of 15 cm and 20 cm between plants and rows, respectively. The length of each row was 3 m. Each genotype had eight rows. The individual plot size was 4.8 m^2. Cultural practices were done as recommended for rice cultivation.

Collection of leaf samples and isolation of pathogen: Rice leaves infected with *Bipolaris oryzae* were collected from the experimental field of NRRP, Hardinath. The leaves of Samba Mahsuri having typical symptom were cut into small pieces of 2 to 3 cm and surface sterilized with 0.1% NaOCl for one minute and rinsed three times with distilled water. The cut pieces were transferred to sterilized glass petri-plates containing two layers of moist blotting paper and incubated in an incubator at 25±1ºC for two days for sporulation. The identification of the pathogen was made by following the method described in a technical bulletin on seed borne disease and seed health testing of rice (Agarwal *et al*., 1989). Single conidium was picked-up from sporulating rice leaf sample and transferred it to rice leaf extract agar plates.

Preparation of media: Rice leaf extract agar media was prepared with the composition of 100 g rice leaves, agar 20 g and dextrose 10 g for 1 liter of final volume in water. The media was autoclaved at 121ºC and 15 psi pressure for 20 minutes and allowed to cool to bring around 50 to 60ºC in room temperature before pouring in sterilized petri-plates and test tubes under the laminar air flow chamber.

Maintenance of *Bipolaris oryzae* pure culture: The pure culture of *Bipolaris oryzae* was maintained by transferring single conidium to rice leaf extract agar slants in test tube and stored in refrigerator below 5ºC.

Spore production: Five petri-plates with rice leaf extract agar was inoculated with *B. oryzae* by taking 5 mm discs of actively growing pathogen from pure culture plates. The inoculated plates were incubated under fluorescent light for 14 days in an incubator.

Pot filling with soil and seed sowing: The experiment was done in February at AFU, Rampur. Plastic pots (6 cm diameter) were filled with sandy loam soil mixed with FYM in the ratio of 2:1 which was sterilized by using 4% formaldehyde. Rice seeds of the test genotypes were sown @15 grains per pot at 1.5 cm apart and covered with thin layer of soil. Pots were watered daily till the end of the experiment.

Preparation of inoculum: The fully grown *B. oryzae* culture plates were flooded with sterile distilled water and allowed to stand for 5 minutes. The culture was scrapped with sterile glass slide. Spore suspension was filtered through the double layered muslin cloth and the concentration of spore suspension was maintained at 4×10^4 spores per ml with sterile distilled water by the help of haemocytometer.

Inoculation of rice seedlings: Inoculation was done on 21 day old seedlings of the test genotypes with the spore suspension of *B. oryzae* using hand sprayer. Pots were kept surrounded by wet gunny bags for 48 hours to maintain conducive environment for infection. The leaf wetness was maintained by spraying water regularly. Three days after inoculation, seedlings were visually examined daily for symptom development.

Disease assessment*:* In glasshouse experiment, disease scoring was done four times at each day starting from three days after inoculation. Disease scoring was done for three times at three days intervals starting from 31 days after seeding, four times at seven days intervals starting from 45 days after transplanting in both nursery and field.

Disease observations were taken using the disease rating scale of 0-9 (IRRI, 2002) as mentioned in Table 1. Visual estimates of leaf area infected were made and disease severity was recorded.

Table 1. Scale for foliar disease assessment (IRRI, 2002)

Score	Infection
0	No incidence
1	Less than 1%
2	1-3%
3	4-5%
4	6-10%
5	11-15%
6	16-25%
7	26-50%
8	51-75%
9	76-100%

In glasshouse, each individual plant in a pot (ten plants per pot) was scored for the disease and each plant in a row was scored for the disease severity in nursery. On transplanted rice, ten hills were selected randomly for each genotypes of each plot and tagged for disease scoring at different time interval to find out the disease severity.

$$\text{Disease severity} = \frac{\text{sum of all numerical rating}}{\text{total no. of samples observed} \times \text{maximum rating}} \times 100$$

Disease severities were calculated per plant and mean severity was computed per plot. AUDPC values were computed, from

the disease severities values using the formula given by Das *et al*. (1992).

$$\text{AUDPC}=\sum_{i=1}^{n}(Y_{i+1}+Y_i)0.5(T_{i+1}-T_i)$$

Where,

Yi = disease scored on the first date
Ti= date on which the disease was scored
n= number of dates on which disease was scored.

Resistance and susceptibility of genotypes: The genotypes were categorized into four categories based on the AUDPC value (Table 2) (Aryal and Shrestha, 2013).

Table 2. Resistant and susceptible categories of genotypes based on mean AUDPC value

Mean AUDPC	Category	Symbol
> 340	Highly susceptible	HS
260-340	Susceptible	S
180-260	Moderately resistant	MR
<180	Resistant	R

The final grain yield was adjusted at 12% moisture level by using following formula (Paudel, 1995).

$$\text{Grain yield} = \frac{(100-\text{MC})\times \text{plot yield (kg)} \times 10000\ \text{m2}}{(100-12)\times \text{plot area}}$$

Where, MC= moisture content of grain in percentage.

Data Analysis: The recorded data were subjected for the analysis of variance and mean separation test was done using Duncan's multiple range tests (Gomez and Gomez, 1984). Data analysis was done using R 3.0.3 (R Core Team, 2013) and the agricolae version 1.1-8 package (De Mendiburu, 2014). Dendrogram with average linkage and euclidean distance was prepared using MINITAB 14.

RESULTS AND DISCUSSION

Area under disease progress curve (AUDPC)

The AUDPC value for all rice genotypes in glasshouse varied significantly at 5, 6 and 7 DAI. Genotypes survived up to eight days after inoculation. The genotypes TOX 894-9-1, Madhaya dhan 845, Makwanpur-1 and Sabitri had the least AUDPC value and the genotypes Lalka Basmati, Sankharika, IR 73008-136-2-2-3, Hardinath-1 and Samba Mahsuri showed higher AUDPC value in glass house. The rice genotypes varied significantly in area under disease progress curve (AUDPC) values in 31 and 34 DAS in nursery. The AUDPC values increased with time of observation in all the genotypes. The AUDPC value was highly variable in the genotypes due to the difference in susceptibility to the pathogen. The genotypes Sabitri and Radha-4 showed the least AUDPC value and the genotypes Sankharika and Samba Mahsuri had higher AUDPC value in nursery. Rice genotypes differed significantly in area under disease progress curve ($P \leq 0.001$) at 52, 59 and 66 DAT and increased with time in field (Fig. 2). The genotypes Sabitri and Radha-4 had lower total AUDPC value and the genotypes Sankharika, TCA 80-4, IR 73008-136-2-2-3, Lalka Basmati and BI 0530-5-10-1-2 had higher total AUDPC value in the field (Fig.2).

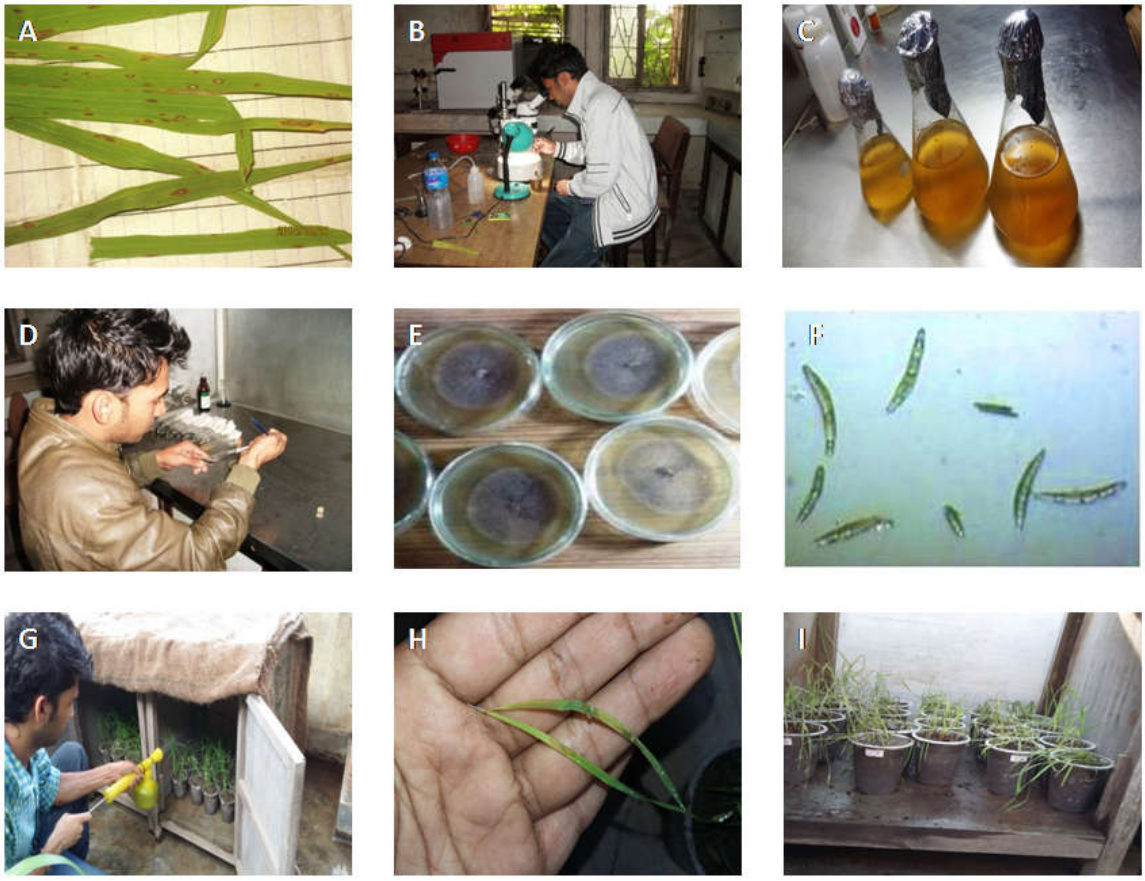

Fig.1. Isolation of *B. oryzae* and reactions tested against 20 rice genotypes. A, rice leaves with typical brown spot; B, single conidium isolation; C, rice leaf extract agar media; D, pure culture; E, spore production; F, conidia; G, inoculation; H, observation of symptom; I, response of rice genotypes

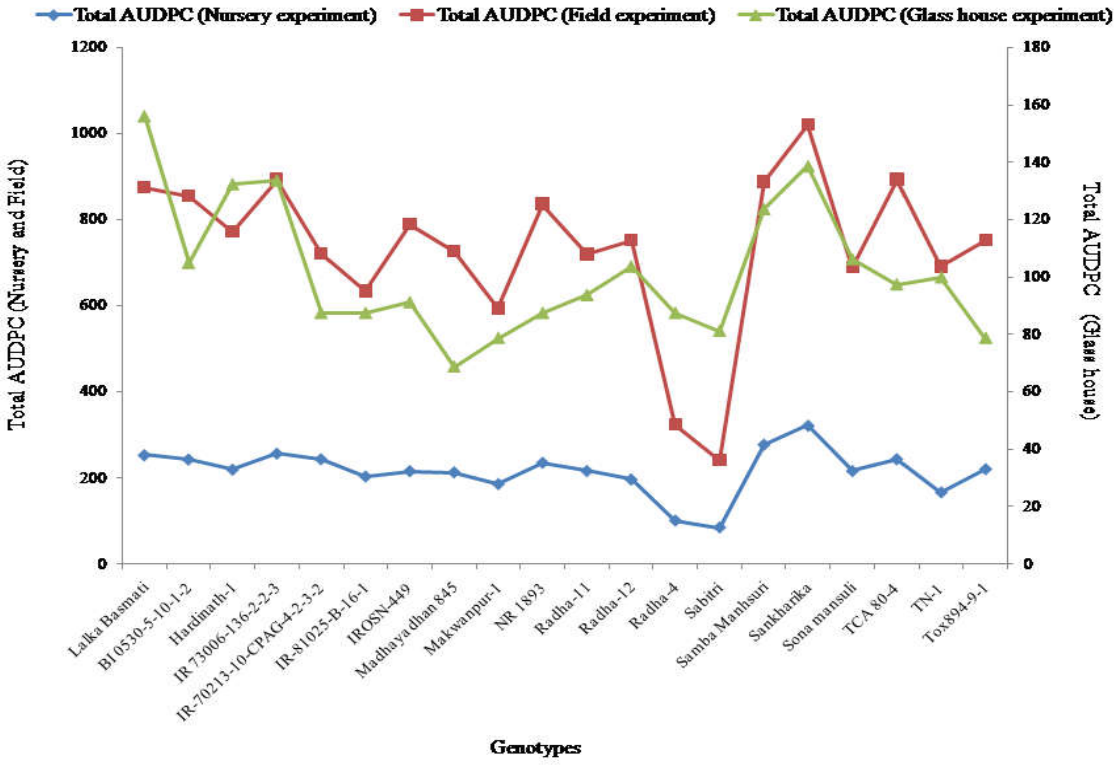

Fig.2. Total AUDPC of 20 different rice genotypes at nursery, glasshouse and field experimentations

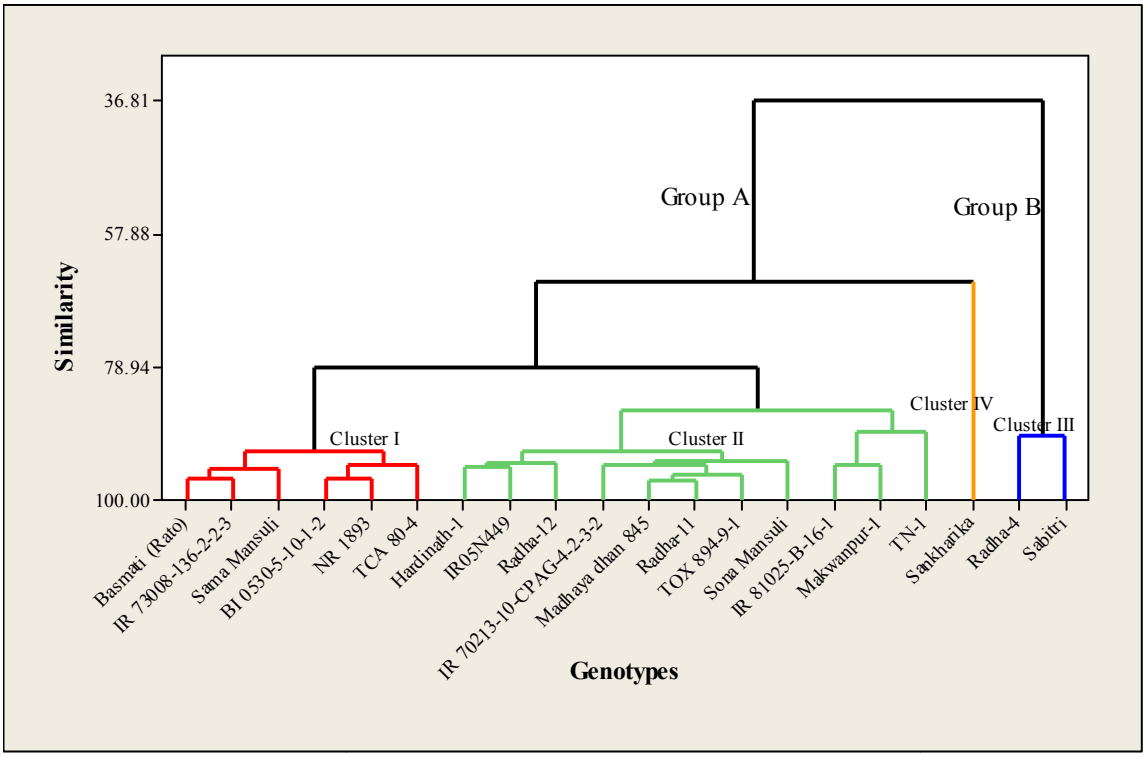

Fig.3. Dendrogram showing similarity amongst 20 rice genotypes according to the UPGMA method based on AUDPC value of *Bipolaris oryzae* in field and glasshouse experiments

The study was carried out to assess the reactions of different rice genotypes in response to *Bipolaris oryzae* and the possible use of these genotypes in breeding programme to manage brown leaf spot. In the field and glasshouse experiments, the twenty test genotypes which were used for screening against *B. oryzae* showed variable responses. Similar variation in rice germplasm in response to brown spot disease was reported by Hossain and Kulkarni (2001); Padmanabhan and Ganguly (1954) reported that multiple foci of infection, prolonged period of favourable environmental conditions and host susceptibility results in epiphytotic conditions. The mean maximum temperature of 32.48ºC, mean RH 85.05% and accumulated rainfall of 708.3 mm in the field was very conducive for rapid disease development. Under natural epiphytotic conditions, three genotypes Sankharika, IR 73008-136-2-2-3 and TCA 80-4 were even more susceptible than the

susceptible check genotype Samba Mansuri. It may be due to the association with compatible races of *B. oryzae* and also due to high inoculum pressure. Radha-4 showed resistance against the pathogen as of Sabitri. It could to attributed resistant in the genes cultivars against *B. oryzae*. Major and minor genes of a host plant contribute towards resistant reactions. Adair (1941) reported that cumulative effect of several recessive genes governs resistance of a host. Balal *et al.* (1979) found two dominant genes associated with resistance and one gene was associated with susceptibility of a host. The maintenance of favourable environment for disease development in glasshouse may have caused rapid disease development and the highest disease pressure in Lalka Basmati, Sankharika, IR 73008-136-2-2-3, Hardinath-1 and Samba Mahsuri showing higher total AUDPC value. The delayed rates of disease development and lower total AUDPC were observed in TOX 894-9-1, Madhaya dhan 845, Makwanpur-1 and Sabitri indicating higher level of horizontal resistance. Thus, screening of these different genotypes revealed that none of them was highly resistant to *B. oryzae*. In general, moderately resistant and resistant genotypes observed in the glasshouse also showed less disease intensity in the field when compared with the susceptible genotypes. Percich *et al.* (1997) reported that infection efficiency of pathogen increases with increase in temperature, humidity and moisture. In our study, increase in AUDPC value of even resistant genotypes in the field might be due to the high inoculum pressure and favourable environment.

Table 3. Resistant and susceptible categories of 20 rice genotypes based on mean AUDPC of *Bipolaris oryzae* grown in field at Hardinath, Dhanusha, 2014/15

Genotypes	Mean AUDPC	Category
Sankharika	340.05 ± 2.63^{a}	Highly susceptible
TCA 80-4	297.71 ± 1.88^{ab}	Susceptible
IR 73008-136-2-2-3	297.71 ± 3.02^{ab}	
Samba Mahsuri	295.98 ± 1.55^{ab}	
Lalka Basmati	291.66 ± 3.43^{ab}	
BI 0530-5-10-1-2	285.18 ± 0.74^{ab}	
NR 1893	278.70 ± 1.49^{a-c}	
IR05N449	263.14 ± 9.08^{b-d}	
Hardinath-1	257.09 ± 1.55^{b-d}	Moderately
Radha-12	250.61 ± 1.14^{b-d}	
TOX 894-9-1	250.61 ± 5.68^{b-d}	
Madhaya dhan 845	241.97 ± 3.11^{b-d}	
IR 70213-10-CPAG-4-2-3-2	241.91 ± 2.63^{b-d}	
Radha-11	239.80 ± 4.16^{b-d}	
TN-1	230.30 ± 2.40^{b-d}	
Sona Mansuli	229.87 ± 5.96^{b-d}	
IR 81025-B-16-1	211.29 ± 6.39^{cd}	
Makwanpur-1	197.89 ± 2.83^{d}	
Radha-4	108.02 ± 1.72^{e}	Resistant
Sabitri	80.36 ± 1.97^{e}	

AUDPC: Area under disease progress curve; values in the columns followed with different alphabetic superscripts are significant at P≤0.001 by Duncan's multiple range test; SEm (±) indicates standard error of mean.

Category of genotypes on the basis of mean AUDPC values

On the basis of field experiment, rice genotypes were categorized into four categories on the basis of mean AUDPC values: resistant, moderately resistant, susceptible and highly susceptible. None of the tested genotypes was found highly resistant on the basis of standard disease rating scale given by IRRI. Out of 20 genotypes, two genotypes were found resistant and 10 genotypes were found moderately resistant to brown spot (Table 3). The results clearly indicated that the resistance and susceptibility of these genotypes are still maintained as reported by earlier workers. Devkota (2014) had also reported the lowest disease severity, mean AUDPC and AUDPC per day in Sabitri (13.457%, 93.331 and 11.11, respectively) and the highest in Samba Mahsuri (19.279%, 129.282 and 15.84, respectively). The resistance of genotypes to brown spot disease suggests that there is potential to reduce the loss caused by brown spot disease by growing resistant to moderately resistant rice genotypes. According to the resistant/susceptible categories, four clusters are formed showing similarity amongst 20 rice genotypes based on AUDPC value in field and glasshouse experiments following principal component analysis and cluster analysis by Mohapatra (2002) (Fig. 3). Susceptible groups were more closely related than that of resistant groups. In cluster 1st, six susceptible genotypes are grouped, which represents 30% of the total genotypes. In cluster 2nd, eleven moderately resistant genotypes are grouped, which represents 55% of the total genotypes. In cluster 3rd, two resistant genotypes are grouped which represents 10% of the total genotypes. In cluster 4th, there is one highly susceptible genotype, which represents 5% of the total genotypes.

Disease development per day

Due to different genetic background, crop stage, prevailing environmental condition, host nutrition difference etc., differential rate of disease development have been recorded in rice genotypes. Initially, the differences in brown spot severity among the rice genotypes were not so pronounced, but over time, it progressed faster in Sankharika and Samba Mahsuri compared to other genotypes. In the nursery, rate of disease progress was slow in Sabitri and Radha-4 compared to other genotypes (Fig. 4). Sabitri had much slow rate of disease progress than that of Radha-4. The slow disease development rate indicates high level of partial resistance in the genotypes. In the field, similar pattern was found that of the nursery (Figure 5). Rate of disease progress was steeper in later stage in the field in the susceptible genotypes. In IR 70213-10-CPAG-4-2-3-2, initially the disease progress was higher but later the rate decreased.

Final disease severity, Grain yield and Thousand grain weight

The rice genotypes varied significantly in yield and yield attributes (P ≤ 0.001) (Table 4). The maximum grain yield was recorded in Sabitri (4.76 t ha^{-1}) followed by Radha-4 (4.63 t ha^{-1}), BI 0530-5-10-1-2 (4.61 t ha^{-1}) and NR 1893 (4.56 t ha^{-1}) respectively. The minimum grain yield was recorded in Sankharika (2.76 t ha^{-1}) and Samba Mahsuri (2.93 t ha^{-1}) (Table 4). Different genotypes have different level of yield potential; however the level of disease might also have influenced the yield. Sankharika and Samba Mahsuri had the lowest grain yield with highest disease severity. Genotypes with low yield might be due to their low yield potential as well as high disease severity. In the present study, rice genotypes showing the maximum disease severity had the least grain yield. High disease severity causes rapid necrosis of the leaf and thereby reducing the chlorophyll content. Sanchote and Van Ba (2005) reported that leaf infection by *B. oryzae* did not significantly affect number of panicles per hill or the number of spikelets per panicle, but caused a decline in yield

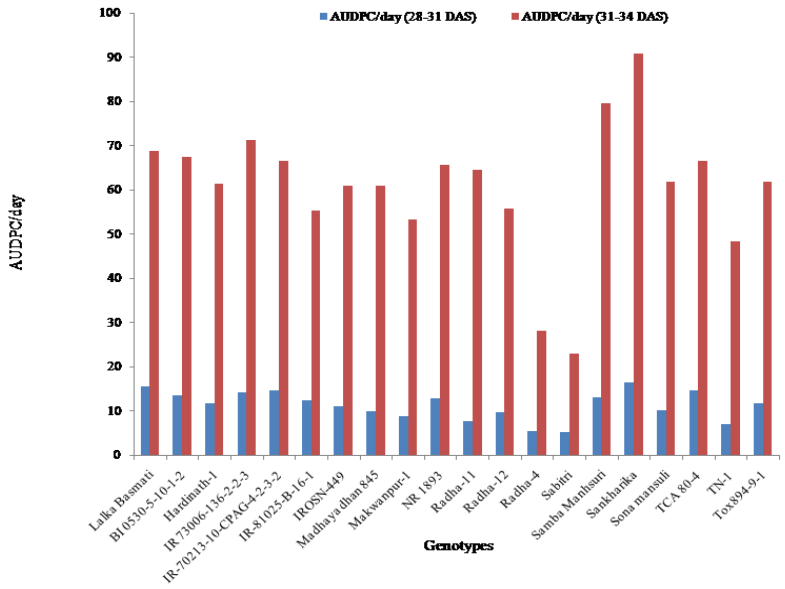

Fig.4. Per day AUDPC of *Bipolaris oryzae* in rice genotypes in the nursery at Hardinath, Dhanusha, 2014/15

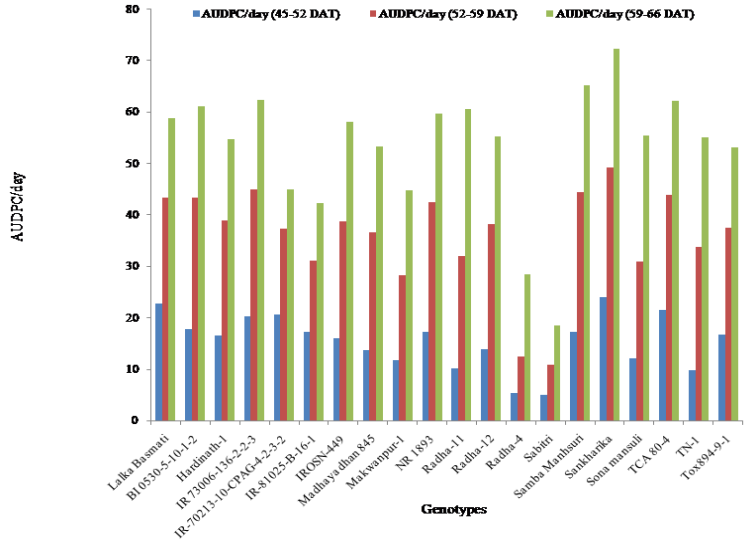

Fig.5. Per day AUDPC of *Bipolaris oryzae* in rice genotypes in the field at Hardinath, Dhanusha, 2014/15

Table 4. Final disease severity, grain yield and thousand grains weight of rice genotypes grown in field at Hardinath, Dhanusha, 2014/15

Genotypes	Final disease severity (66 DAT)	Grain yield (t ha^{-1})	1000 grain weight (g)
NR 1893	62.96	4.56 ± 0.03^{a}	22.19 ± 0.04^{de}
BI 0530-5-10-1-2	64.44	4.61 ± 0.07^{a}	$23.51 \pm 0.02^{c-e}$
Radha-4	37.77	4.63 ± 0.12^{a}	22.17 ± 1.12^{de}
Sabitri	21.85	4.76 ± 0.12^{a}	25.88 ± 0.47^{bc}
Sona Mansuli	69.62	$3.33 \pm 0.17^{f-h}$	$21.26 \pm 0.29^{d-f}$
Makwanpur-1	49.25	4.23 ± 0.08^{b}	27.35 ± 0.13^{b}
IR 73008-136-2-2-3	65.92	$3.63 \pm 0.08^{c-e}$	22.35 ± 1.08^{de}
Madhaya dhan 845	57.77	3.83 ± 0.09^{c}	22.02 ± 0.05^{de}
Radha-12	57.03	3.76 ± 0.10^{cd}	$20.71 \pm 1.12^{e-g}$
Lalka Basmati	62.22	$3.33 \pm 0.12^{f-h}$	$21.19 \pm 0.10^{d-f}$
IR70213-10-CPAG-4-2-3-2	49.62	$3.46 \pm 0.12^{e-g}$	24.04 ± 0.72^{cd}
TCA 80-4	67.40	3.15 ± 0.02^{hi}	22.34 ± 1.08^{de}
Radha-11	71.48	$3.50 \pm 0.05^{e-g}$	25.93 ± 0.06^{bc}
Sankharika	77.77	2.76 ± 0.08^{j}	18.33 ± 0.33^{gh}
Samba Mahsuri	65.92	2.93 ± 0.08^{ij}	16.85 ± 1.38^{h}
Hardinath-1	59.25	$3.40 \pm 0.05^{e-h}$	21.65 ± 0.79^{de}
TOX 894-9-1	58.88	3.23 ± 0.07^{gh}	$18.60 \pm 0.17^{f-h}$
IR 81025-B-16-1	49.62	$3.56 \pm 0.08^{d-f}$	24.12 ± 1.73^{cd}
IR05N449	62.96	$3.31 \pm 0.04^{f-h}$	31.29 ± 2.65^{a}
TN-1	57.03	$3.30 \pm 0.05^{f-h}$	22.29 ± 1.6^{de}

Figures in the columns followed with alphabetic superscripts are significant at P≤0.001 by Duncan's multiple range tests;
SEm (±) indicates standard error of mean

by increasing the number of empty grains, reducing the number of grains per panicle and grain weight. The highest grain yield was recorded in Sabitri and Radha-4 due to less disease severity. These two genotypes were also consistently resistant in all the observation dates. The genotypes Sabitri and Radha-4 had the least final disease severity. Thus, they had higher grain yield and the genotype Sankharika had the least grain yield due to the highest final disease severity. Although, BI 0530-5-10-1-2 and NR 1893 had higher final disease severity, the grain yields of these genotypes were statistically similar with Sabitri and Radha-4.

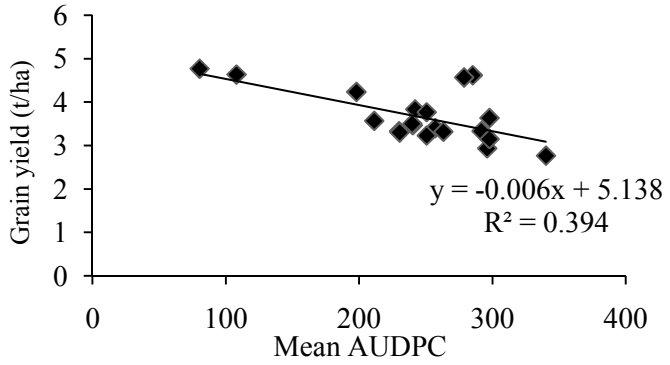

Figure 6. Estimated linear relationship between mean AUDPC and grain yield of 20 rice genotypes at Hardinath, Dhanusha, 2014/15

Regression study

There was significant ($P \leq 0.01$) negative linear relationship between mean AUDPC and grain yield (Figure 6). According to the coefficient of determination, about 39.4% variation in grain yield was due to mean AUDPC. Higher temperature combined with high disease severity in the field affects the grain filling that ultimately cause reduction in 1000 grain weight and yield (Duveiller *et al*., 2005).

Conclusion

The genotypes Sabitri and Radha-4 showing resistance against brown spot disease can be utilized as a source of resistance for breeding and for cultivation by farmers of Dhanusha and similar conditions. The genotypes like BI 0530-5-10-1-2 and NR 1893 had higher disease incidence and disease severity but the grain yield was statistically similar with high yielders and the most resistant genotypes Sabitri and Radha-4. So, they are potential candidates for multi-location trial.

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