Dissecting parameters associated with sheath rot (*Sarocladium oryzae* [(Sawada) W. Gams & D. Hawksw]) disease in rice (*Oryza sativa* L.)

C. Mahadevaiah[1], M. K. Prasanna Kumar[2] and Shailaja Hittalmani[1,]*

[1]Marker Assisted Selection Laboratory, Department of Genetics and Plant Breeding, and
[2]Department of Plant Pathology, University of Agricultural Sciences, Bengaluru 560 065, India

Accurate measurement of parameters contributing to diseases and their symptoms is important for identification of resistant genotypes. Present sheath rot scoring system such as disease incidence scoring system accounts for only percentage of disease incidence and not the disease severity. Disease severity scoring system is based on combined features of panicle exertion and lesion size, very laborious and less accurate in field condition. Existing disease index requires assigning of incidence-severity scores for each and every tillers in a plant and has greater difficulties in evaluation of large number of plants or breeding pools. Hence, we propose phenotypic parameters such as proportion of panicle exertion, proportion of diseased and healthy sheath and modified disease severity scoring system for accurate measurement of disease. A high-throughput and simple disease indices (severity index, lesion index, detached lesion index and panicle discolouration index) are proposed for accurate identification of resistant-susceptible genotypes.

Keywords: Dissecting parameters, *Oryza sativa*, *Sarocladium oryzae*, sheath rot.

SHEATH rot disease caused by *Sarocladium oryzae* [(Sawada) W. Gams & D. Hawksw] has emerged as one of the major diseases in almost all rice-growing ecosystems of the world. It is a serious menace to rice cultivation, causing yield losses of 3–85% depending on disease severity[1–3] and complete suppression of panicle exertion[4]. The fungus is internally and externally seed borne, survives in plant debris[5], weeds and soil. Secondary infection is due to wind or insect borne[2,3] and pathogen infects through flag leaf sheath. Pathogen produces phytotoxins, viz. cerulenin and helvoic acid[6–9] and whole genome sequencing of *S. oryzae* from our group revealed genes involved in production of cerulenin and helvoic acid[10]. These phytotoxins are responsible for production of greyish-brown necrotic lesion in flag leaf sheath and restricts translocation of photosynthates to the developing panicles, causing quantitative (chaffy grains) and qualitative yield loss including discolouration of grains, reduced seed viability, nutritional and market value[8,11]. Incidence of sheath rot disease is highly correlated with sucking pests[12], cooler climate with high humidity[13,14] and moisture stress during reproductive stages in aerobic condition.

Accurate measurement of parameters contributing to diseases and their symptoms is important for identification of resistant genotypes. Sheath rot disease incidence (SES)[15] and severity scores[16] used for measurement of disease are inadequate to identify the resistant genotypes[17]. The existing 'SES' scoring system accounts for only percentage of infected tillers and not for the severity of the disease. Severity scoring is based on combined features of lesion size and extent of panicle exertion. The severity scores are assigned to each and every tiller (1–9 scale), viz. small lesion with normal panicle exertion (score-1), enlarged lesion with normal panicle exertion (score-3), 6–15% of diseased sheath with 75% panicle exertion (score-5), 16–50% diseased sheath with 50% of panicle exertion (score-7) and higher than 50% of diseased sheath with less than 25% of panicle exertion (score-9). Assigning severity scores to every tillers, estimation of percentage of diseased sheath from spindled flag sheath and disease index[16] are difficult and laborious for large number of plants. Hence, in this study, phenotypic parameters, viz. proportion of panicle exertion, proportion of diseased and healthy flag leaf sheath are proposed for accurate disease measurement. Simplified sheath rot severity scoring system and novel disease indices (severity index, lesion index, detached lesion index and panicle discolouration index) are proposed for identification of sheath rot disease resistance in rice.

New phenotypic parameters, viz. proportion of panicle exertion, proportion of healthy and diseased flag sheath were estimated from averaged values from five tillers per plant. Panicle length[15], panicle exertion[15], flag leaf sheath length (measured from top most node to ligule of flag leaf at physiological maturity stage) and sheath rot lesion length (measured lesion length longitudinally on flag leaf sheath at physiological maturity stage) were used for estimation of new parameters as follows.

Proportion of panicle exertion

$$= \frac{\text{Panicle length (cm)} + \text{panicle exertion (cm)}}{\text{Panicle length (cm)}},$$

Proportion of diseased flag leaf sheath

$$= \frac{\text{Sheath rot lesion length (cm)}}{\text{Flag leaf sheath length (cm)}},$$

Proportion of healthy flag sheath

$$= \frac{\text{Flag leaf sheath length} - \text{sheath rot lesion length (cm)}}{\text{Flag leaf sheath length (cm)}}.$$

*For correspondence. (e-mail: shailajah_maslab@rediffmail.com)

Simplified disease severity scores were assigned to each plant based on percentage of diseased sheath (% of disease sheath = proportion of disease sheath averaged over five tillers per plant × 100) and categorized into severity scores (1–9 scale) (Table 1).

SES[15] scores converted into percentage of infected tillers and severity parameters (severity scores, lesion length) were averaged across five tillers used for estimation of disease indices.

Severity index = mean percentage of infected tillers × mean sheath rot severity scores.

Lesion index = mean percentage of infected tillers × mean sheath rot lesion length.

Varietal response to sheath rot disease was categorized into different response groups based on modified disease indices value[16] as immune (0), highly resistant (1–100), resistant (101–200), moderately resistant (201–300), moderately susceptible (301–500), susceptible (501–700) and highly susceptible (>700).

In vitro screening protocols, viz. standard grain inoculation[17], detached sheath assay and electrolyte leakage assay[18] and mycelial insertion into sheath[9] were used for screening of rice genotypes for sheath rot disease. Detached tiller assay[19] is very simple, accurate and suitable to assess absolute resistance of breeding pool or genotypes under laboratory conditions. Fewer number of tillers per plant are sufficient to represent absolute resistance and rest of tillers could be used for field screening/yield parameters. Therefore, two *in vitro* based indices, viz. detached lesion index and panicle discolouration index were proposed.

Detached lesion index = mean percentage of infected tillers × mean sheath rot lesion length by detached tiller assay.

Panicle discolouration index = mean percentage of infected tillers × mean length of panicle discolouration by detached tiller assay method.

The existing and proposed disease indices were compared using selected recombinant inbred lines (RILs), parents (BPT5204 and HP14) and check (IR64) during *kharif* season, 2013. Disease response of different genotypes was consistent among genotypes for immune and highly resistant responses (Table 2). Severity and lesion indices were highly effective in differentiating susceptible and highly susceptible genotypes compared to existing disease index as observed in HPR81, HPR141, HPR658 and IR64 genotypes due to two reasons. First, severity and lesion indices were estimated based on mean values of each plant, whereas assigning scores to each tillers in existing scoring system, the tillers with disease escape or lesser disease severity sums up to error or lesser disease index value. Secondly, severity and lesion indices estimated based on average lesion length were more accurate and realistic compared to existing disease index estimated from visually scored parameters. Therefore, new proposed severity and lesion indices are more accurate compared to existing and *in vitro* based detached lesion and panicle discolouration indices. *In vitro* based disease indices showed considerable variation for disease response as influenced by turgidity of sheath and genotypic variation for nodal roots developed during the incubation period[20].

Field experiments consisting of 276 RILs, parents (BPT5204 and HP14) and checks (IR64, MAS26, MAS99 and MAS946-1) were evaluated for sheath rot disease under artificial disease screening during *kharif* season, 2014 (July sowing) and late *kharif* season 2014 (October sowing) under aerobic condition. The experiments were conducted in randomized complete block design with two replications in experimental plots, Department of Genetic and Plant Breeding, UAS, GKVK, Bengaluru.

Artificial disease screening was adopted to enrich the inoculum by different methods[19], viz. seeds soaked in conidial suspension (10^5 conidia/ml) overnight, whole plant inoculation with cotton swab method (10^5 conidia/ml) during peak vegetative stage and, foliar inoculations (10^5 conidia/ml) from peak vegetative to booting stages. Significance of mean for sheath rot disease attributing traits between *kharif* and late *kharif* was tested using *t* test assuming unequal variances at 5% level of significance.

Tall statured parent HP14 recorded maximum panicle exertion during both *kharif* (5.25 cm) and late *kharif* (3.88 cm) with average panicle exertion of 126% and 120% respectively. No disease incidence was observed and was highly resistant to sheath rot disease based on disease indices (Table 3). Semi dwarf statured BPT5204 recoded partial panicle exertion (–3.94 cm and –8.56 cm) with average panicle exertion of 69% and 38% during *kharif* and late *kharif* seasons respectively. Maximum disease incidence was observed in BPT5204 and categorized as highly susceptible based on severity and lesion indices, and moderately susceptible based on detached lesion and panicle discolouration indices.

Table 1. Disease severity scores

Diseased flag leaf sheath (%)	Severity scores
Less than 1%	1
2–5% of flag leaf sheath infected	3
6–25% of flag leaf sheath infected	5
26–50% of flag leaf sheath infected	7
More than 50% of sheath infected	9

Table 2. Comparison of disease indices among selected RILs and parental lines

RIL	Existing disease index	Proposed indices: Severity index	Lesion index	Detached lesion index	Detached panicle discolouration index
HPR-64	0 (I)	0 (I)	0 (I)	0 (I)	0 (I)
HPR-81	590 (S)	700 (S)	900 (HS)	425 (MS)	450 (MS)
HPR-117	15 (HR)	75 (HR)	19 (HR)	202 (MR)	188 (R)
HPR-141	162 (R)	600 (S)	675 (S)	450 (MS)	738 (HS)
HPR-221	0 (I)	0 (I)	0 (I)	0 (I)	0 (I)
HPR-229	558 (S)	700 (S)	833 (HS)	450 (MS)	667 (S)
HPR-235	0 (I)	0 (I)	0 (I)	0 (I)	0 (I)
HPR-377	12 (R)	8 (R)	11 (R)	49 (R)	55 (R)
HPR-616	661 (S)	800 (HS)	1300 (HS)	617 (S)	508 (S)
HPR-618	358 (MS)	633 (S)	733 (HS)	558 (S)	1200 (HS)
HPR-658	447 (MS)	833 (HS)	1367 (HS)	1450 (HS)	1250 (HS)
HPR-817	716 (HS)	800 (HS)	1100 (HS)	667 (HS)	1100 (HS)
HPR-867	506 (S)	650 (S)	675 (S)	933 (HS)	1263 (HS)
HP-14	0 (I)	0 (I)	0 (I)	0 (I)	0 (I)
BPT-5204	763 (HS)	850 (HS)	975 (HS)	421 (MS)	479 (MS)
IR-64	452 (MS)	780 (HS)	1340 (HS)	1275 (HS)	1125 (HS)

Table 3. Mean performances of parameters related to sheath rot disease resistance in 276 RILs derived from BPT-5204 × HP-14 cross under artificial disease screening in rice

	BPT5204		HP-14		RILs (mean)	
Traits	*Kharif*	Late *Kharif*	*Kharif*	Late *Kharif*	Kharif	Late *Kharif*
Plant height (cm)	37.96	37.96	107.28	78.23	89.68**	65.88**
Panicle exertion (cm)	–3.94	–8.56	5.25	3.88	1.47**	–4.83**
Proportion of panicle exertion	0.69	0.38	1.26	1.20	1.07**	0.68**
Sheath rot lesion length (cm)	11.37	10.56	0.00	0.00	3.75**	7.55**
Proportion of healthy sheath	0.52	0.37	1.0	1.0	0.85**	0.61**
Proportion of diseased sheath	0.61	0.63	0.00	0.00	0.17**	0.39**
Sheath rot (SES) scores	8.33	9.00	0.00	0.00	2.08**	4.17**
Sheath rot severity scores	7.88	8.25	1.00	1.00	2.81**	5.53**
Infected tillers (%)	95.83	100	0	0	20.50**	38.03**
Sheath rot lesion length (cm) under detached tillers assay	5.75	4.21	4.81	3.15	6.20	6.04
Panicle discolouration (cm) under detached tillers assay	10.38	4.79	8.25	3.00	9.22**	7.28**
Severity index	758.33	825.00	0.00	0.00	159**	298**
Lesion index	1089.86	1056.25	0.00	0.00	292**	451**
Detached lesion index	557.29	420.83	0.00	0.00	125**	239**
Panicle discolouration index	997.92	479.17	0.00	0.00	212**	328**
Grain yield/plant (g)	21.30	3.13	11.87	2.83	18.21**	3.31**

*Significant at 1%; **Significant at 5%.

Significant difference was observed for mean performance of RILs for sheath rot disease attributing traits between *kharif* and late *kharif* seasons. Sheath rot disease incidence and severity, proportion of diseased sheath, percentage of infected tillers and disease indices were high during late *kharif* season compared to regular *kharif* season, suggesting environmental influence in the expressions of disease. *Kharif* season recorded higher mean for panicle exertion compared to late *kharif*. Micro environmental features such as cooler temperatures coupled with high humidity[14] prevalent during August to November and partial panicle exertion[20,21] favour the disease development.

Estimates of phenotypic correlation coefficients showed significant positive correlation between newly proposed phenotypic parameters and disease indices with sheath rot scoring system. Proportion of diseased sheath and disease indices showed positive significant correlation with present scoring system (Table 4). Proportion of panicle exertion and plant height recorded significant negative correlation with disease incidence (SES)[20,21], disease severity and disease indices. Significantly high

Table 4. Estimates of phenotypic correlation coefficients for sheath rot disease resistance-attributing traits in 276 RIL's derived from BPT-5204 × HP-14 cross in rice

Traits	PH	PE	PrPE	ShLL	PrHSh	PrDSh	ShR (SES)	ShR (S)	PIT	ShLLD	PLD	DI-1	DI-2	DI-3	DI-4
PH	1.000														
PE	0.576**	1.000													
PrPE	0.605**	0.969**	1.000												
ShLL	–0.521**	–0.814**	–0.777**	1.000											
PrHSh	0.601**	0.810**	0.795**	–0.978**	1.000										
PrDSh	–0.601**	–0.810**	–0.795**	0.978**	–1.000**	1.000									
ShR (SES)	–0.627**	–0.680**	–0.679**	0.671**	–0.704**	0.703**	1.000								
ShR (S)	–0.535**	–0.699**	–0.690**	0.723**	–0.738**	0.738**	0.760**	1.000							
PIT	–0.688**	–0.647**	–0.664**	0.661**	–0.707**	0.706**	0.818**	0.555**	1.000						
ShLLD	–0.022	–0.022	–0.047	0.122**	–0.135**	0.135**	0.094*	0.052	0.111**	1.000					
PLD	–0.219**	–0.226**	–0.248**	0.292**	–0.316**	0.316**	0.236**	0.214**	0.255**	0.572**	1.000				
DI-1	**–0.706****	**–0.660****	**–0.684****	**0.713****	**–0.763****	**0.763****	**0.780****	**0.573****	**0.975****	**0.124****	**0.275****	1.000			
DI-2	**–0.627****	**–0.640****	**–0.651****	**0.778****	**–0.803****	**0.803****	**0.727****	**0.552****	**0.917****	**0.148****	**0.285****	**0.960****	1.000		
DI-3	–0.551**	–0.539**	–0.564**	0.586**	–0.628**	0.627**	0.690**	0.462**	0.838**	0.448**	0.411**	**0.830****	**0.806****	1.000	
DI-4	–0.606**	–0.562**	–0.596**	0.609**	–0.656**	0.656**	0.694**	0.488**	0.860**	0.314**	0.510**	**0.867****	**0.841****	**0.904****	1.000

*Significant at $P = 0.05$, **Significant at $P = 0.01$. PH, Plant height (cm); PE, Panicle exertion (cm); PrPE, Proportion of panicle exertion; GY, Grain yield/plant (g); ShLL, Sheath rot lesion length (cm); PrHSh, Proportion of healthy sheath; PrDSh, Proportion of diseased sheath; ShR (SES), ShR disease incidence scores (SES scale); ShR (S), ShR severity scores; PIT, Percentage of infected tillers (SES scale); ShLLD, Sheath rot lesion length under detached tiller assay; PLD, Panicle discolouration under detached tiller assay; DI-1, Severity index; DI-2 , Lesion index; DI-3, Detached lesion index; DI-4, Panicle discolouration index.

positive association was observed among the disease indices (Table 4), suggesting that any of the four disease indices are useful for accurate measurement of sheath rot disease and identification of resistant genotypes.

In the present study, new proposed disease indices were more accurate in measurement of disease resistance and identification of resistant genotypes compared to existing disease index. *In vitro* based detached lesion and panicle discolouration indices were relatively more variable due to the turgidity and genotypic variation for nodal roots developed during incubation period. Therefore, severity and lesion indices derived from the mean values of disease incidence and severity parameters were more accurate in differentiating resistant-susceptible genotypes and useful in crop improvement breeding for disease resistance in rice.

1. Chen, M. J., Studies in sheath rot of rice. *J. Agric. Forestry Taiwan*, 1957, **7**, 84–102.
2. Amin, K. S., Sharma, B. D. and Das, C. R., Occurrence of sheath rot of rice in India caused by *Acrocylindrium*. *Plant Dis. Rep*., 1974, **58**, 358–360.
3. Chakravarthy, D. K. and Biswas, S., Estimation of yield loss in rice affected by sheath rot. *Trans Br. Mycol. Soc*., 1978, **62**, 226–227.
4. Raina, G. L. and Singh, G., Sheath rot outbreak in Punjab. *Int. Rice Res. Newsl.,* 1980, **5**(1), 16.
5. Milagrosa, S., Transmission of *Sarocladium oryzae* (Sawada) W. Gams & Hawksv through rice seeds. Ph D thesis, University of the Philippines, Los Banos, Philippines, 1987.
6. Shakthivelu, N. and Gnanamanickam, S. S., Isolation of and assay of cerulenin produced by rice sheath rot pathogen *Sarocladium oryzae* (Swada). *Curr. Sci*., 1986, **55**(19), 988–989.
7. Tschen, L. C., Shean-Tzong, H. and Wu, T. W., Isolation and phytotoxic effects of helvolic acid from plant pathogenic fungus *Sarocladium oryzae*. *Bot. Bull. Acad. Sin.*, 1997, **38**, 251–256.
8. Ghosh, M. K., Amudha, R., Jayachandran, S. and Sakthivel, N., Detection and quantification of phytotoxic metabolites of *Sarocladium oryzae* in sheath rot-infected grains of rice. *Lett. Appl. Microbiol*., 2002, **34**, 398–401.
9. Nandakumar, R., Babu, S., Amutha, G., Raguchander, T. and Samiyappan, R., Variation in toxin production among isolates of *Sarocladium oryzae*, the rice sheath rot pathogen. *Plant Path. J*., 2007, **6**, 120–126.
10. Hittalmani, H., Mahesh, H. B., Mahadevaiah, C. and Prasannakumar, M. K., *De novo* genome assembly and annotation of rice sheath rot fungus *Sarocladium oryzae* reveals genes involved in helvolic acid and cerulenin biosynthesis pathways. *BMC Genomics*, 2016, **17**, 271; doi:10.1186/s12864-016-2599-0.
11. Gopalakrishnan, C., Kamalakannan, A. and Valluvaparidasan, V., Effect of seed-borne *Sarocladium oryzae*, the incident of rice sheath rot on rice seed quality. *J. Plant Protect. Res*., 2010, **50**(1), 98–102.
12. Lakshmanan, P., Mohankumar, S. and Velusamy, R., Role of *earhead bug* (*Leptocorisa acuta*) feeding on *sheath rot disease caused by Sarocladium oryzae in rice* (*Oryza sativa L.*) *in India. Phytoparasitica*, 1992, **20**(2), 107–112.
13. Reddy, M. M., Reddy, C. S. and Reddy, A. G. R., Influence of season and planting date on sheath rot incidence ID rice. *Indian Phytopathol*., 1999, **52**(3), 289–290.
14. Reddy, M. M., Reddy, C. S. and Reddy, A. G. R., Influence of weather parameters and insect pest populations on incidence and development of sheath rot of rice. *Indian Phytopathol.*, 2001, **54**(2), 179–184.
15. IRRI, Standard evaluation system for rice, Rice Knowledge Bank, 2002; www.knowledgebank.irri.org
16. Narayanasamy, P. and Viswanathan, R., A new scoring system for sheath rot of rice. *Madras Agric. J*., 1990, **77**(5–6), 256–257.
17. Madhav, M. S., Latha, G. S., Padmakumari, A. P., Somasheakar, N., Mangruthia, M. N. and Viraktamath, B. C., Phenotyping rice for molecular plant breeding. In *Phenotyping for Plant Breeding: Applications of Phenotyping Methods for Crop Improvement* (eds Kumar, P. S. and Kumar A. A.), Springer, 2013, pp. 15–16.
18. Samiyappan, R. *et al*., Purification and partial characterization of a phytotoxin produced by *Sarocladium oryzae*, the rice sheath rot pathogen. *Arch. Phytopathol. Plant Protect*., 2003, **36**, 247–256.
19. Mahadevaiah, C., Shailaja, H., Uday, G. and Prasanna Kumar, M. K., Standardization of disease screening protocol for sheath rot disease in rice. *Int. J. Agric. Sci. Res*., 2015, **5**(5), 129–135.
20. Srinivasachary, Shailaja, H., Girish Kumar, K., Shashidhar, H. E. and Vaishali, M. G., Identification of quantitative trait loci associated with sheath rot resistance (*Sarocladium oryzae*) and panicle exsertion in rice (*Oryza sativa* L.). *Curr. Sci.*, 2002, **82**(2), 133–135.
21. Hittalmaini, S., Girishkumar, S. K., Bagali, P. G., Srinivasachary and Shashidar, H. E., Identification of QTLs associated with sheath rot resistance in two mapping populations of rice (*Oryza sativa* L.). In Fourth International Rice Genetics Symposium, Los Banos. *Rice Genetic Newsletter*, 2000, vol. 16, pp. 87–88.

Received 8 July 2015; revised accepted 10 August 2016

doi: 10.18520/cs/v112/i01/151-155

Manganilmenite in the magnetite ore body from Pokphur area of Nagaland, North East India and the possibility of microdiamonds in the ophiolites of Indo-Myanmar ranges

Bibhuranjan Nayak[1,3]* and Franz Michael Meyer[2]

[1]Mineral Processing Division, CSIR-National Metallurgical Laboratory, Jamshedpur 831 007, India
[2]Institute for Mineralogy and Economic Geology, RWTH Aachen University, D-52056 Aachen, Germany and German-Mongolian Institute of Resource Technology (GMIT), 2nd Khoro, Nalaikh District, Ulaanbaatar, Mongolia
[3]Present address: CSIR-Institute of Minerals and Materials Technology, Bhubaneswar 751 013, India

Manganilmenite is found to be associated with the magnetite ore body of Pokphur area in the Nagaland ophiolites, North East India. There is perhaps no earlier description of the mineral from the Indian subcontinent. It occurs as an accessory mineral with magnetite and Fe-chlorite (chamosite). Electron probe micro-analytical data reveal that the mineral contains 5.6–8.5 wt% MnO and traces of MgO, ZnO and Cr_2O_3, while the TiO_2 content remains within narrow limits of 50–53 wt%. The calculated pyrophanite

*For correspondence. (e-mail: brn69@rediffmail.com)