A chlorophyll reading based method for non-destructive screening and scoring of rice genotypes for resistance to bacterial blight

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ABSTRACT

Accurate phenotyping is an essential prerequisite for resistance gene identification and selection of resistant genotypes. Screening for bacterial blight disease infestation and resistance gene identification in rice are based on visual observations, which are error prone. A non-destructive method for screening of resistant genotypes using SPAD chlorophyll meter is described. Two indices, A and B were developed. Both the indices have high correlation with visual selection based standard evaluation system (SES) method. Index B was found more suitable for differentiation between resistant and susceptible lines.

Keywords: Bacterial blight, screening, chlorophyll meter, standard evaluation system, index

Bacterial blight of rice, caused by Xanthomonas oryzae pv oryzae (Ishiyama) Swings *et al.*, is a pandemic disease affecting rice crop all over the world. It is considered as the second most important disease of rice in India next to rice blast and can cause up to 50% crop loss (Gnanamanickam *et al.*, 1999). The disease produces yellow on leaves with wavy margins, which later turn gray. Due to the non-localized disease development pattern the disease generally develops from the top of the leaf progressing downward as well as from the margins to the interior of the leaf in a characteristic wavy pattern. In natural infection, disease inoculum may come from seed, stubbles of infected plants, other weed hosts and spread through the help of wind resulting in either random or uniform distribution of disease (Mundt *et al.*, 1999). The disease is difficult to control by chemical means, thus breeding for resistance is the best option to fight against this disease. Availability of an appropriate scoring method is of paramount importance to screen natural populations of rice for resistance, to demarcate the boundary between resistance and susceptibility, to monitor disease progress

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in the fields and to study the epidemiology of the disease. International Rice research Institute (IRRI), Los Banos, Philippines has developed a standard evaluation system (SES) for rice, which advocates the use of a qualitative screening of disease on the basis of visual inspection of diseased area in the rice leaf (IRRI, 1996). This method is based on a 1 – 9 scale, where the score '1' stands for 0 – 3% infected area and the score '9' stands for 95 – 100% infected area. Problem of visual inspection is that the accuracy of scoring depends heavily on the evaluator, due to the wavy disease progress pattern (Jain *et al.,* 1983). The SES converts a quantitative expression into qualitative scores, making statistical interpretation less robust, which is less suitable for precise phenotyping. In most genetic analysis, robust phenotyping is essential for identification of resistance genes and QTLs controlling the phenotype. The genetic studies on the inheritance of resistance to bacterial blight in rice often use another screening system developed by Kaufmann *et al.,* (1973). It is based on inoculation by clipping flag leaf or top 2 – 3 leaves with scissors dipped in a solution containing the bacteria. Once the disease develops through the wounds, the downward advance of disease after 15 – 21 days is recorded by a scale. The disease development is very slow in resistant lines progressing less than 5 cm, while in susceptible lines the disease progresses beyond 15 – 25 cm. This method is most common for identification of rice genotypes resistant to bacterial blight. However, variation in the number of days after which scoring is to be done and disputes over the demarcating boundary for resistance and susceptibility is a major bottleneck in this method (Sahu and Khush, 1988; Ogawa, 1993; Satya *et al.,* 2004). Moreover, this method may not be very suitable for study of disease progress, and is limited to screening by artificial inoculation. The method cannot be applied in natural infection, where the actual time of disease development is not known.

In the following section a screening method based on the loss of chlorophyll content of rice leaves is described. The new method is based on loss of chlorophyll content in leaves upon disease infection. As the disease progresses, it causes yellowish to grayish lesion with cell death. However, due to structural integrity of monocot leaves the cytoskeletal architecture of the leaf is not destroyed, only the leaf turns yellow to gray due to loss of chlorophyll and cell death. The loss of chlorophyll content due to disease development can, therefore, be recorded and used as a stable indicator for disease progress. Thus disease progress can be better estimated by determination of chlorophyll content. Spectrophotometric methods of estimation of chlorophyll content can not be used for taking multiple measurements on the same leaf, thus a non-destructive method is required for estimation of chlorophyll content. Such nondestructive estimation of chlorophyll content is possible through the use of a Chlorophyll Meter, which can be used for obtaining rapid and accurate multiple measurements from the same leaf. A protocol for estimation of disease progress is described here for rapid screening of rice genotypes exhibiting resistance to bacterial blight. The method is suitable for monitoring both natural disease development and disease development after artificial inoculation in rice.

Materials and Methods

Susceptible rice cv. Swarna was grown with three replications in two blocks of size 25 square meters. Standard management practices were adopted for raising a healthy crop. Adequate supply of nitrogenous fertilizer was ensured by using the leaf colour chart at the vegetative stages, so that yellowing due to nitrogen deficiency does not interfere with the screening procedure. Natural infection was noticed early in the vegetative stages of crop development. A portable chlorophyll meter (Minolta SPAD meter) was used to measure the chlorophyll content in terms of SPAD (Soil Plant Analysis Development) value in the fully developed control and infected leaves. In every alternate leaf measurement, the instrument was calibrated for obtaining accurate results. The fully grown top 3-4 leaves except flag leaf were taken for measurement of SPAD value. Each leaf was graded in ten to fifteen equal grids depending on total leaf length and three random readings were taken in each grid (Figure 1). Average SPAD value was determined in infected and control leaves and indices were developed by using the following formula:

Index A = [(SPAD control – SPAD infected)/ SPAD control] x 100

Index B = [Index A x lesion length at margin]/ days to score after inoculation or disease initiation

Average SPAD value was determined in different infection stages corresponding to the SES scoring scale. For

construction of index B, lesion length was measured after 21 days, as this is mostly used in genetic analyses.

Table 1: Average SPAD values, Index A, Index B and their relationship with SES scores

SES scale	Infected area (%)	SPAD value	Index A	Lesion length (cm)	Index B
1	0 – 3.0	45.2	6.80	0.8	0.26
2	4.0 – 6.0	43.6	10.10	3.1	1.49
3	7.0 – 12.0	35.8	26.19	7.9	9.85
4	13.0 – 25.0	26.9	44.54	13.6	28.84
5	26.0 – 50.0	18.6	61.65	28.0	82.20
6	51.0 – 75.0	14.7	69.69	41.0	136.06
7	76.0 – 87.0	7.8	83.92	44.0	175.83
8	88.0 – 94.0	3.9	91.96	46.9	205.37
9	95.0 – 100.0	1.2	97.53	47.2	219.20
Mean	-	21.97	54.71	25.83	95.46
SD	-	16.76	34.54	19.62	90.37

Table 2: Correlation among different indices

	SPAD value	Index A	Lesion length	Index B
Infected area	-0.95**	0.95**	0.95**	0.99**
SPAD value		-1.00**	-0.98**	-0.97**
Index A			0.98**	0.97**
Lesion length				0.98**

Results and Discussion

Chlorophyll meter measures the light transferred through a leaf to predict the amount of chlorophyll and a corresponding value SPAD is used to indicate the content of chlorophyll. Use of chlorophyll meter is widely recognized in the management of nitrogenous fertilizers of rice, because nitrogen is a key component of chlorophyll (Balasubramanian *et al.*, 2000; Stevens 2006). As bacterial blight causes de-greening of leaf tissues in a progressive and non-localized manner, it was postulated that this may be effectively used for screening for bacterial blight resistance. Although there are reports of assessing loss from pest and disease infestation by using a chlorophyll meter, there is possibly no report of using this as a technique for screening rice populations and no standard methodology is yet available.

For determining the average SPAD value of the leaves, each leaf was divided into 10 - 15 small grids. Within each grid, three random SPAD measurements were taken, thus a total of 30 – 45 SPAD values were calculated for each leaf (Figure 1). Index A was developed by the proportionate difference of SPAD value in control and infected leaves. Initially, the infection of bacterial blight was identified through yellowing of leaf tips. To confirm the presence of bacterial blight, the leaf tissues were incubated in peptone water for 24 h and the solution was cultured in yeast chalk glucose agar (YCGA) medium specific for Xanthomonas for 7 days at 28°C. The Xanthomonas colonies were identified by characteristic yellowish white raised colonies. During the initial stage of disease development, leaves were tagged

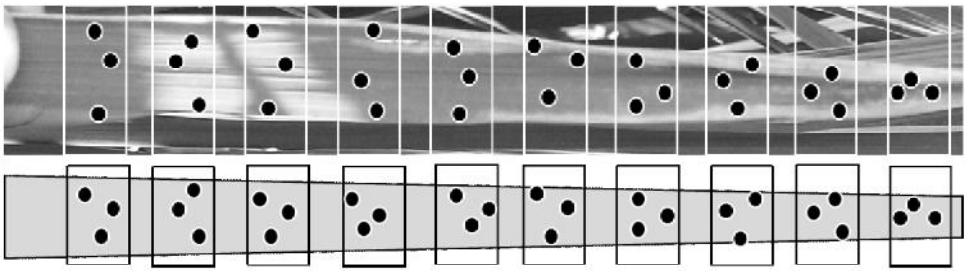

Fig. 1: Grided observation of disease infestation using SPAD meter

Fig. 2. Relationship between indices A, B and SES score

for measurement and disease progress was monitored daily at 4:00 PM in the tagged leaves. To compare SES and SPAD scores, SPAD scores were taken along with corresponding SES scores and lesion length. The SPAD values ranged from 45.2 at corresponding SES stage 1 to 1.2 at corresponding SES stage 9 (Table 1). The index A was determined to be 6.8 at SES stage 1 and 97.53 at SES stage 9. The SES score based on area infected was highly correlated with index A (r = 0.95** – 0.98**) (Table 2).

Another index, B was constructed to accommodate the number of days after which scoring is done. As there are major differences in published reports on the number of days after which disease is to be scored, a check can be applied by introducing this parameter. It was observed that index B was highly correlated with index A as well as the SES scores (Table 2). Both the indices are, therefore, are in accordance with the SES system. As expected, SPAD values and Index A showed no difference in correlation pattern, because correlation is independent of change in scale and origin. However Index A is positively correlated to SES scores, while the orientation of SPAD values is opposite. Moreover, Index A provides the scope of comparative estimation including the control, so is a better estimator of disease scoring than SPAD value itself.

For genetic analysis and identification of resistance genes, it is essential to classify this continuous scale into two classes, as most of the resistance genes for bacterial blight have been found to be monogenic dominant or recessive in nature. Classification on the basis of SES has not been used in most genetic analyses. While Sahu and Khush (1988) recommended a plant having < 10 cm lesion length to be resistant, Shanti *et al.,* (2001) considered plants with < 4 cm lesion length as resistant only. The indices proposed here may serve as a better alternative in classifying resistant and susceptible genotypes. From the results obtained, it is proposed that plants having a value of < 50 for Index A and Index B may be considered as resistant and those with higher values may be classified in the susceptible group (Figure 2). The difference between resistant and susceptible genotypes for index B is higher compared to difference for index A. The high class difference between resistant and susceptible group will help to more clearly identify resistant lines. A SES score value beyond 4 indicates the susceptibility of a genotype. The difference between indices A and B also increases beyond SES score 4, while the resistant lines have similar values for indices A and B. Index B provides a higher range than Index A. It includes all the factors area infected, lesion length and disease scoring interval. The disease scoring interval itself is an important factor, which is highly influenced by the environmental factors and geographical locations. Use of index B thus may be more suitable for genetic analysis as well as epidemiological studies over different locations.

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References

1. Balasubramanian, V., Morales, A.C., Cruz, R.C., Thiyagarajan, T.M., Nagarajan, R., Babu, M., Abdulrachman, S. and Hai, L.H. 2000. Adaptation of the chlorophyll meter (SPAD) technology for real time N-management in rice : a review. *International Rice Research Notes* **25**: 4-8.
2. Gnanamanickam, S.S., Priyadarisini, V.B., Narayanan, N.N., Vasudevan, P. and Kavitha, S. 1999. An overview of bacterial blight disease of rice and strategies for its management. *Current Science* **77**: 1435-1443.
3. IRRI 1996. Standard evaluation system for rice. International Rice Research Institute, Laguna, Los Banos, Philippines.
4. Jain, R.K., Shukla, S.N., Dath, A.P. and Devadath, S. 1983. Enumerators' scoring ability - a possible source of introducing variation in varietal reaction to bacterial blight of rice. *Indian Phytopathology* **36**: 163-164.

5. Kaufmann, H.E., Reddy, A.P.K., Hseih, S.P.Y. and Merca, S.D. 1973. A improved technique for evaluating resistance of rice varieties to Xanthomonas oryzae. *Plant Disease Reports* **57**: 537-541.
6. Mundt, C.C., Ahmed, H.U., Finckh, M.R., Nieva, L.P. and Alfonso, R. F. 1999. Primary Disease Gradients of Bacterial Blight of Rice. *Phytopathology* **89**: 64-67.
7. Satya, P., Singh, V.P. and Singh, A.K. 2004. Genetic analysis of pyramided resistance genes for resistance to bacterial blight of rice (Xanthomonas oryzae pv. oryzae) and development of resistant lines of Basmati rice (Oryza sativa). *Indian Journal of Agricultural Sciences* **75**(*7*) : 428-431.
8. Stevens, G. 2006. Use of a portable chlorophyll meter to manage crop nitrogen in rice. www.muextension.missouri.edu/expolr/
9. Sahu, R.K. And Khush, G.S. 1988. Genetic analysis of bacterial blight resistance in rice. *International Rice Research Notes* **13** : 14-15.
10. Ogawa, T. 1993. Methods and strategy of monitoring race distribution and identification of resistance genes to bacterial leaf blight (Xanthomonas oryzae pv. oryzae) in rice. *Japanese Agricultural Research Quarterly* **27**: 71-80.
11. Shanti, M.L., George, Vera, M.L.C., Cruz C.M., Bernardo M. A., Nelson, R.J., Leung, H., Reddy, J.N. and Sridhar, R. 2001. Identification of resistance genes effective against rice bacterial blight pathogen in Eastern India. *Plant Disease* **85**: 506-512.