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Short Communication

Image-based phenotyping of spot blotch disease symptoms in wheat varieties

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Abstract
We investigated the use of computer-assisted image analysis techniques for the classification and scoring of spot blotch symptoms in wheat. We compared the symptoms developed in resistant and susceptible genotypes for disease. Ning 8139 and Agra Local were selected as resistant and susceptible genotypes. We analyzed images of diseased leaves to get information about types of symptoms developed on resistant and susceptible genotypes after artificial inoculation. And found that Ning 8139 and Agra Local produced sixteen and forty spots with thirteen and fifty percent of average disease severity. We also observed that in Agra Local spots were surrounded by yellow halo and several spots coalesce with each other and formed big lesion, which were absent in Ning 8139.

Keywords: Spot Blotch, Image, Phenotyping, Wheat, Symptoms

1. Introduction
Spot blotch caused by *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus* is one of the most important foliar diseases limiting wheat production in warmer, non-traditional growing areas (Chowdhury *et al*., 2013) [3]. *Bipolaris sorokiniana* having a hemibiotrophic life style, in which it act as a biotrophs in early phase of infection and later convert to nectrophs in this phase it secretes various toxin to kill their host cell (Schafer *et al*., 2004) [7]. A few resistant genotypes have been reported as resistance till date by various workers but no genotypes are reported immune to spot blotch disease. Genotypes viz., Chirya 1, Chirya 3, Chirya 7, Jinmai 4058, Ning 8201, Suzhoe etc were reported as resistant and genotypes viz., Milan, HD2662, Longmai-10, WH 542 etc reported as moderately resistant (Sharma *et al*., 2004; Joshi *et al*., 2004) [9, 5]. Thus, the present investigation was undertaken on spot blotch of wheat caused by *B. sorokiniana* to obtain information on the difference in types of symptoms produced in resistant and susceptible genotypes using image analysis software Image J.

Wheat genotype Agra Local and Ning 8201 were used as susceptible and resistant plants. And *Bipolaris sorokiniana* strain BS112 was used for artificial inoculation on wheat plants in green house. Pathogen were mass multiplied on sorghum seeds and incubated at 26°C for fifteen days (Jeyarajan 2006; Upadhyay and Mukhopadhyay, 1986) [4, 10]. Pathogen spores were isolated using muslin cloth and sprayed on sixty-nine days old plants. Plants were irrigated and covered with polythene sheet for 36 hours to maintain sufficient humidity to facilitate disease development. Seven days after artificial inoculation leaves sample were taken. Using HP scanner leaves were scanned and images were captured. Computer software Image J were used for image analysis which is a public domain, java based image processing program developed at National Institute of Health (Schneider *et al*., 2012) [8].

Bipolaris sorokiniana is known to develop symptom in all plant parts i.e. internodes, stem, nodes, leaves, awn, glumes and seed and disease is known as seedling blight, node cankers, black point and spot blotch when its appear on seedling, node, seeds and leaves respectively (Zillinsky 1983) [11]. Wheat genotype Ning 8201 was reported as resistant for spot blotch pathogen B. sorokiniana (Neupane *et al*., 2007) [6]. But we have observed that virulent strain of B. sorokiniana BS112 develop symptom in both resistant and susceptible parent in artificial inoculation, but there were difference in types of symptom they produced. Leaves sample were collected seven days after artificial inoculation and examined using computer based software Image J. We have observed that lesions on resistant leaves were developed as small, dark

brown lesions, ~1.07 mm long, without a chlorotic margin (Figure 1; Table 1). In susceptible genotypes, these lesions extend quickly ~5.7 mm and form oval to elongated light brown to dark brown several centimeter blotches that coalesce and result in the death of the leaf tissues (Figure 1; Table 1). Yellowing surrounding the necrotic area is more prominent as well as half of leaf shows necrosis in susceptible genotypes, rather limited or no yellow halo and necrosis of leaf tip is hardly 3-5 mm in resistant genotypes as it is shown in Figure 1.

We counted number of spots on both resistant and susceptible genotypes using Image J software, and data reveals that on an average forty and sixteen spots appeared on Agra Local and Ning 8201 respectively. Using Image J software first total area of leaves were calculated and then diseased area were calculated (Figure 2) and in Microsoft excel percentage disease severity were calculated. The data reveals that in Ning 8201 its range from 10-15 and average of 13%, but in Agra Local its range from 25-69 and average of 50%. Adlakha *et al*., 1984 developed 0-5 rating scale for foliar disease. Comparison of Image J data with disease ratting scale its revels that Ning 8201 and Agra local falls under 2 and 4 scales respectively.

A digital image analysis method previously used to evaluate leaf color changes due to nutritional changes, remote sensing, detecting, quantifying and classifying plant diseases (Barbedo 2013) [1]. Phenotyping using Image analysis software has several advantages viz. it is both reliable and accurate. If a good automated system can be developed then it can be extremely powerful. Image analysis equipment is relatively inexpensive. But in this early stage of science and technology it has some disadvantages viz. coping with plant-to-plant variation in color and various image artifacts or flaws is not straightforward. It is not yet established how to deal with multiple diseases, damage or physiologic conditions on sample leaves. It requires some training in the program to become proficient. Validation is often required to ensure the quality of the measurement (Bock *et al*., 2010) [2]. As far as our result is concerned Image J software can be used for disease classification and screening of segregating population viz. F2, RIL.

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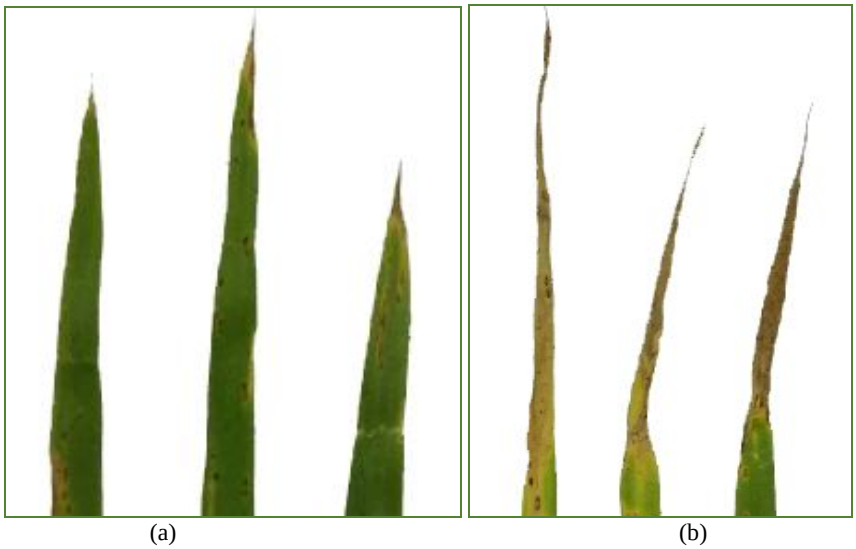
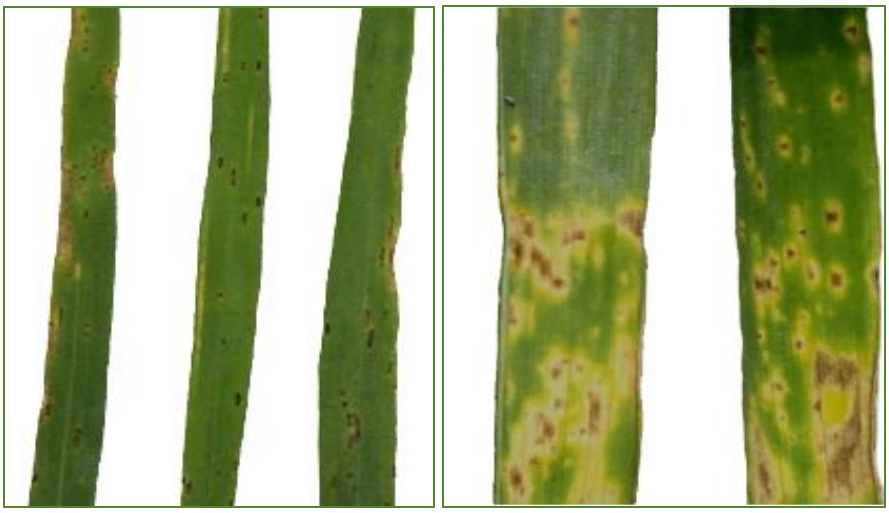


(a) (b)

Fig 1: Necrosis of leaf tip on (a). Resistant genotype (Ning 8201); (b). Susceptible genotype (Agra Local)



(a) (b)

Fig 2: Types of spot on (a). Resistant genotype (Ning 8201); (b). Susceptible genotype (Agra Local)

Table 1: Effect of *Bipolaris sorokiniana* on leaves of Ning 8201 and Agra Local

S. No	Total Area (cm^2)	Diseased Area (cm^2)	Percentage (%)	No of Spot per leaf	Average spot length per leaf (cm)
Susceptible Leaves					
1	4.4	1.6	35.4	23	0.65
2	5.6	1.4	25.3	33	0.56
3	11.6	5.4	46.6	35	0.43
4	7.4	3.3	44.6	29	0.41
5	7.9	4.5	57	25	0.38
6	9.3	6.4	68.8	45	0.77
7	10.8	6.7	62	65	0.55
8	12.3	7.1	57.7	30	0.59
9	15.4	7.9	51.3	73	0.79
10	13.6	6.8	50	40	0.63
Average	10	5	50	40	0.567
Resistant leaves					
1	17.5	2.6	14.9	30	0.15
2	11.4	1.5	13.2	24	0.09
3	14.3	2.1	14.7	22	0.1
4	16.5	2.4	14.5	15	0.13
5	6.6	0.9	13.6	8	0.1
6	12.5	1.3	10.4	17	0.08
7	8.6	1.2	14	14	0.09
8	10.2	1.2	11.8	11	0.11
9	15.2	2.3	15.1	13	0.12
10	7.5	0.8	10.7	8	0.1
Average	12	2	13	16	0.107

References

1. Barbedo JGA. Digital image processing techniques for detecting, quantifying and classifying plant diseases. Springer Plus, 2013; 2(1):660.
2. Bock CH, Poole GH, Parker PE, Gottwald TR. Plant disease severity estimated visually, by digital photography and image analysis, and by hyperspectral imaging. Critical Reviews in Plant Sciences, 2010; 29(2):59-107.
3. Chowdhury AK, Singh G, Tyagi BS, Ojha A, Dhar T, Bhattacharya PM. Spot blotch disease of wheat–a new thrust area for sustaining productivity. Journal of wheat research. 2013, 5(2).
4. Jeyarajan R. Prospects of indigenous mass production and formulation of Trichoderma, pp 74-80. In Current Status of Biological Control of Plant diseases using antagonistic organisms in India (Eds Rabindra RJ Ramanujam B) Project Directorate of Biological Control, Bangalore, 2006, 445.
5. Joshi AK, Kumar S, Chand R, Ortiz-Ferrara G. Inheritance of resistance to spot blotch caused by Bipolaris sorokiniana in spring wheat. Plant Breeding, 2004; 123:213-219.
6. Neupane RB, Sharma RC, Duveiller E, Ortiz-Ferrara G, Ojha BR, Rosyara UR *et al*. Major gene controls of field resistance to Spot Blotch in wheat genotypes 'Ning 8201/Shanghai# 7'and 'Chirya. 3'. Plant Disease, 2007; 91(6):692-697.
7. Schäfer P, Hückelhoven R, Kogel KH. The white barley mutant albostrians shows a supersusceptible but symptomless interaction phenotype with the hemibiotrophic fungus Bipolaris sorokiniana. Molecular plant-microbe interactions, 2004; 17(4):366-373.
8. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to Image J: 25 years of image analysis. Nature methods. 2012; 9(7):671.
9. Sharma RC, Duveiller E, Gyawali S, Shrestha SM, Chaudhary NK, Bhatta MR. Resistance to Helminthosporium leaf blight and agronomic performance of spring wheat genotypes of diverse origins. Euphytica, 2004; 139:33-44.
10. Upadhyay JP, Mukhopadhyay AN. Biological control of Sclerotium rolfsii by Trichoderma harzianum in Sugarbeet. Tropical Pest Management, 1986; 32:215-20.
11. Zillinsky FJ. Common diseases of small grain cereals. A guide to Identification. Mexico DF, Mexico, CIMMYT, 1983.