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Research Article

Evaluating vegetation indices for precision phenotyping of quantitative stripe rust reaction in wheat

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Abstract

Wheat production and productivity is widely affected by stripe rust infection. Resistance to this disease governed by additive-gene effect is one of the recent strategies being adopted in wheat breeding programme. While the present phenotyping approaches for scoring, the plants reaction often vary from person to person. A novel, repeatable and reliable approach can enhance the efficiency of determining genetic variability in large number of genotypes. Taking into consideration the emerging non-invasive tools to assess the physiological status of plants. The present investigation was undertaken to explore utility of optical measurements to variation of the stripe rust reaction in wheat genotypes. One hundred and twenty Indian wheat genotypes representing released varieties, elite genotypes, genetic stocks, and local landraces were used for the study. The stripe rust epidemics in the field were initiated with *Yr27*-virulent *P. Striiformis* race 78S84. The Area under the Disease Progress Curve (AUDPC) values were calculated from four weekly visual estimates of disease severity which ranged from 0 to 2077. Normalized difference vegetation index (NDVI), Chlorophyll content index (CCI) and Plant Canopy temperature (CT) were recorded twice, 7 days apart, when disease severity approached maximum values on the susceptible controls. The results indicate that the temporal ground-based NDVI is most effective in studying quantitative rust reaction with a significant regression coefficient (r^2=0.63) between AUDPC and NDVI data followed by chlorophyll content index (r^2=0.37) and canopy temperature (r^2=0.21).

Keywords: Stripe rust, wheat, normalized difference vegetation index, chlorophyll content index, canopy temperature

1. Introduction

Wheat is one of the most important staple food crops of the world, occupying 17 per cent (one sixth) of crop acreage worldwide, feeding about 40 per cent of the world population and contributing 20 per cent of total food calories and proteins to human nutrition (Reynolds *et al.*, 2001). Stripe rust causes severe losses ranging from 10 to 70 per cent (Chen, 2005), which is mainly due to reduction in photosynthetic area leading to both reduced grain yield and quality (Devadas *et al.*, 2009). A stepped reflectance pattern with low reflectance in the visible and high reflectance in the near infrared is shown by photosynthetically active plant components, primarily leaves (Rahman and Ahmed, 2008) due to chlorosis, presence of coloured pustules or other such symptoms. Various studies have reported the relation between spectral reflectance and rust infected plants (Devadas *et al.*, 2009; Huang *et al.*, 2007, 2012; Moshou *et al.*, 2004; Zhang *et al.*, 2011). Improvements are required in both precision and speed of disease phenotyping in the field, which is not possible through visual scoring due to limitation of availability of sufficiently skilled persons able to differentiate small minor gene based rust disease response and also sometimes faulty angle of capturing the data by human eye as against using optical sensor based measurements which does not have these limitations. This has led to global interest in finding simple and cost-effective optical means for remote sensing of disease severity.

Normalized difference vegetation index (NDVI) is used extensively to monitor crop health status and studies related to nutrient requirements. It is a measure of the normalized difference in reflectance of red and near infrared wavelength bands. NDVI data obtained from hyperspectral imaging of plants and its correlation with various factors like plant diseases (Apan *et al.*, 2004; Bravo *et al.*, 2003; Devadas *et al.*, 2009; Du *et al.*, 2004; Franke & Menz. 2007; Huang *et al.*, 2007, 2012; Jacobi & Kuhbauch, 2005; Kumar *et al.*, 2010; Moshou *et al.*, 2004; Sankaran *et al.*, 2010; Zhang *et al.*, 2011), yield predictions (Balaghi *et al.*, 2008), biomass (Janin *et al.*, 2009; Van Der Meer *et al.*, 2000), plant stress (Carter and Knapp, 2001), etc. has been reported by several authors. Transpiration and the metabolic health of the plant can be measured by canopy temperature (CT). Several studies have related canopy temperature as an index to screen the plants under stress such as drought (Bahar *et al.*, 2011; Gonzalez-Dugo *et al.*, 2005; Leinonen& Jones., 2004; Mohammadi *et al.*, 2012; Moayedi *et al.*, 2011).

Leaf chlorophyll content index (CCI) has been widely used as a trait to screen genotypes under different conditions like nutrition availability (Bojovic and Stojanovic, 2005) leaf nitrogen concentration (Bojovic and Markovic, 2009; Wang *et al.*, 2012) etc. Since, stripe rust affects plant's photosynthetic activity due to breakdown of chlorophyll pigments (Penueles *et al.*, 1994) it can act as an index for quantitative detection of rust at early stages. Jing *et al.* (2007) reported the relation between wheat stripe rust and chlorophyll content of the plant.

There could be two types of remote sensing devices- satellite based and ground based. Most of the studies to date are based on the spatial passive reflectance using satellite data. However, many factors like atmospheric conditions, satellite geometry and calibration, scale of observation, soil backgrounds and nature of crop canopy influence the measurements (Holben, 1986; Justice *et al.*, 1991; Soufflet *et al.*, 1991). Also, the spacial resolution for satellite based remote sensore lies in the range of 2.5 to 30mt, as against vehicle mounted green seeker having 0.6m resolution (Ortiz *et al.*, 2011). Therefore, for such experiments the resolution would be limitation if spacial sensors are considered. Solution to these problems may be active hand held optical sensors which can record data for small plots which is not possible with satellite based data acquisition. The handheld sensor provides sufficient resolution especially considering the small plot size in the present experiment was found very appropriate for capturing canopy reflectance using these sensors. Recent developments in handheld optical sensor technology (West *et al.*, 2003) offers new opportunities for high throughput quantitative assessment of rust reactions under field conditions. Hence, the present study was carried out to assess the utility of three physiological parameters, viz., NDVI, CT, and CCI for efficiently determining genetic variation in stripe rust reaction under field conditions.

2. Materials and methods

2.1 Plant material & field experiment: The present study was conducted at the experimental farm of Directorate of Wheat Research, Karnal (29°42′ N, 77°02′ E) in the Indo-Gangetic Plain in northwestern India, with mildly alkaline sandy loam (Typic Ustochrept) soil. Field trial was carried out in November to mid of April. On the basis of genotyping using SSR marker and pedigree analysis, a set of 120 diverse wheat genotypes comprising released varieties, elite genotypes, genetic stocks and local landraces were selected. Genotypes were obtained from the germplasm unit of the Directorate of Wheat Research, Karnal.

Planting was done in lattice design with three replications. Plot size of $0.15m^2$ consisted of three rows, with uniform spacing of 15cm between them. For each genotype, 54 seeds were alloted to each plot and a total of 18 healthy plants per plot were maintained for analysis by removing the rest. Recommended dosage of fertilizer and irrigation scheduling was followed to ensure a healthy crop (Paliwal *et al.*, 2012). No fungicides were applied.

2.2 Inoculation and assessment of disease severity: For creation of epiphytotic conditions for stripe rust, a highly susceptible Indian wheat land-race Agra Local was chosen as suseptible check and inoculated with *Yr27* gene virulent race *78S84* of *Puccinia striiformis* at the 2-3 leaf stage (Zadok secondary growth stage Z12-14) (Zadok *et al.*, 1974) according to the National Plant protection Standard (Li *et al.*, 1989). Infector rows of susceptible cultivars at an equal distance of 25 cm within the experimental blocks to ensure the stripe rust infection homogeneity in the field trials.

Visual disease scores for stripe rust were recorded four times at weekly interval after the first appearance of disease, starting from primary growth stage 3 corresponding to stem elongation (Zadok *et al.*, 1974). Close observations were taken to monitor/take care of damage caused by biotic stresses other than stripe rust. Stripe rust scores were recorded combining the disease severity and the infection type/s as in the modified Cobb's scale (Peterson *et al.*, 1948). The Area Under the Disease Progress Curve (AUDPC) was calculated for each cultivar over time using the formula:

$$AUDPC = \sum_{i=1}^{n} 1/2\,(Y_i + Y_{i-1})\,(X_i - X_{i-1})$$

where Y_i = rust severity at the i^{th} observation, X_i = time (d) at the i^{th} observation, n = total number of observations, and $Y_0 = X_0 = 0$.

Observations for all the three physiological parameters were recorded by using handheld instruments, twice at weekly interval starting from Primary Stage 5 of Zadok's scale (Heading), when crop showed symptoms of maximum infection.

2.3 Normalized difference vegetation index: The data for NDVI was recorded using optical handheld GreenSeeker sensor (Trimble Industries, Inc.). The value of NDVI gets computed from reflectance measurements in the red (around 660±10 nm) and near infrared (around 780 ±10 nm) portion of the spectrum (Bijay Singh *et al.*, 2011) by using a patented technique to measure the fraction of the emitted light in the sensed area that is returned to the sensors as crop reflectance:

$$NDVI = (R_{NIR} - R_{Red}) / (R_{NIR} + R_{Red})$$

where $\mathbf{R}_{NIR}$ is the reflectance of NIR radiation and $\mathbf{R}_{Red}$ is the reflectance of red radiation. When held at a distance of approximately 0.6–1.0 m from the illuminated surface, instrument senses a 0.6 x 0.1 m^2 area which remain approximately constant over the range of height. The sensor was passed over the crop at a height of approximately 0.9 m above the crop canopy and oriented so that the 0.5 m sensed length was perpendicular and centered to the row. Sensor height above the ground increased proportionally with the advancing stage of growth.

2.4 Canopy temperature: HTC MT4 Infrared handheld thermometer was used to take the readings of canopy temperature. Instrument was held approximately 50cm above the canopy, at an angle of 30 degree from the horizon and 0.5-1 m from the edge of plot. Also the measurements were taken between 11 and 16h when the field site had a warm climate with minimal interference due to wind. Long wave infrared radiations are emitted by plant canopy which are function of its temperature. The infrared thermometer converts these radiations into electrical signal after sensing these radiations. These electrical signals are displayed as canopy temperature. Three readings were taken for each plot across three replications, mean value of which was finally taken for analysis.

2.5 Leaf chlorophyll content: Leaf chlorohyll content measurements were taken by using handheld Plant chlorophyll meter, LEAF+ (FT GREEN LLC). The readings actually taken was the chlorophyll content index. Two frequencies of light one at a wavelength of 660 nm (red) and one at 940 nm (infrared) are emitted by the meter. Leaf chlorophyll absorbs red light but not infrared, the difference in absorption is measured by the meter. Three readings for three random leaves from three random plants were measured and averaged in each plot.

3. Results and discussion

Visual readings for disease score were taken four times at weekly interval, starting at stem elongation (Zadoks primary stage 3). Depending upon the genetic variation in susceptibility, the disease severity initially increased and then gradually reached a plateau. The Area Under the Disease Progress Curve (AUDPC) was computed for each genotype and it varied from 0 to 2077. Table.1 shows the distribution of AUDPC score, with almost equal number of genotypes found in all categories except in AUDPC range of 1-100 having maximum number (55) of genotypes.

Optical measurements were recorded twice at weekly interval at the time when crop showed symptoms of maximum infection of stripe rust relative to the susceptible check. Mean value was calculated for indices falling under different AUDPC range (Table.1).

Maximum value for NDVI (0.76), CCI (47.49) and minimum value for CT (23.15) was observed in the category where genotypes showed no symptoms of stripe rust. Where as, genotypes in the higher range of AUDPC, had low NDVI and CCI and high CT. Values for NDVI (0.64) and CCI (42.58) decreased in susceptible lines and was minimum in genotypes with maximum AUDPC. Similarly, CT variation was obtained with maximum (24.38) value in the highest AUDPC range.

Table 1. Mean value and standard error of indices of NDVI, canopy temperature and chlorophyll content index for different categories of genotypes based on AUDPC range

AUDPC Range	No. of genotypes	NDVI	Canopy temperature	Chlorophyll content index
0	14	0.76±0.005	23.15±0.15	47.49±0.57
1-100	55	0.73±0.003	23.35±0.08	46.56±0.30
101-200	12	0.71±0.005	23.46±0.18	47.14±0.96
201-500	13	0.73±0.006	23.42±0.21	44.54±1.03
501-1000	13	0.67±0.006	23.89±0.27	43.35±1.00
>1000	13	0.64±0.006	24.38±0.18	42.58±0.64

3.1 Quantitative analysis: Simple Linear Regression analysis was carried out to examine the relation of three indices to the AUDPC in wheat varieties (Table.2). The trend and regression equations obtained for AUDPC with NDVI and CCI are shown in Figure 2a & b.

Data revealed that AUDPC was inversely proportional to both NDVI (r = -0.81) and CCI (r = -0.61) while directly proportional to CT. This could be attributed to physiological damage caused by stripe rust in terms of chlorophyll degradation and altered concentration of other pigments. Adverse implications of stripe rust on physiology of plants due to chlorophyll degradation (Penueles *et al.*, 1994) and with alteration in the concentration of other pigments i.e. carotenoids and anthocynins (Young & Britton, 1990; Gitelson *et al.*, 2001).

Table 2. Coefficient of determination and correlation coefficient between AUDPC and the three measurement indices

Vegetation Index	Coeff. of determination (r^2)	Correlation coefficient index
NDVI	0.66***	-0.81
Canopy temperature (CT)	0.21***	0.46
Chlorophyll content index (CCI)	0.26***	-0.51

** *Statistically significant at 0.001*

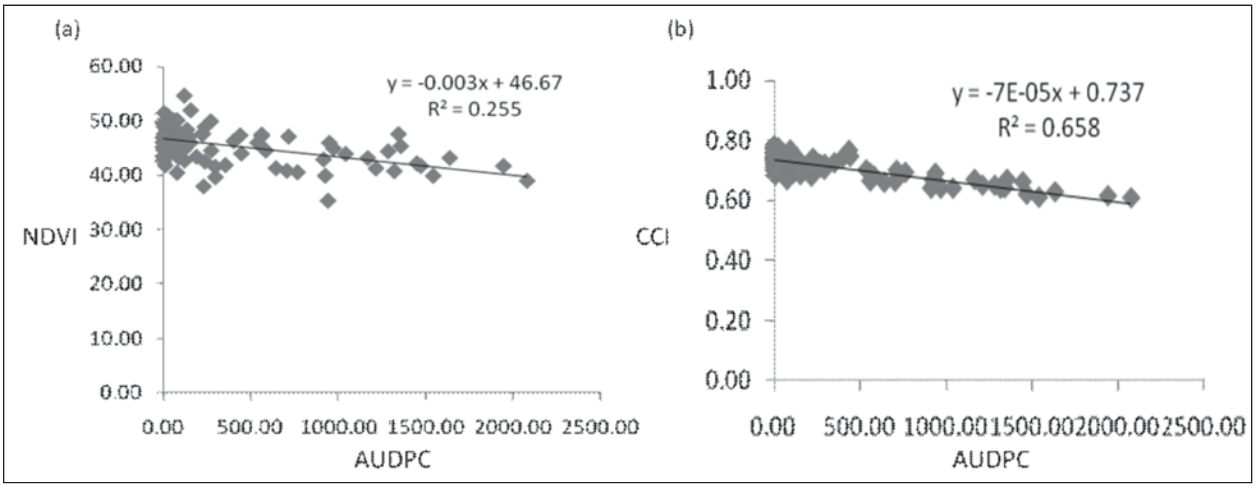

Fig 2. Plot of AUDPC as a function of (a) Normalized difference vegetation index; (b) Chlorophyll content index

Chlorophyll pigment breakdown, during stripe rust infection was also reported by Jing *et al.*, 2007 in winter wheats where they quantified chlorophyll 'a' concentrations.

Various researchers (Bravo *et al.*, 2003; Devadas *et al.*, 2009; Huang *et al.*, 2007, 2012; Moshou *et al.*, 2004; Zhang *et al.*, 2011) have documented the relationship between NDVI measurements and rust severity using spatial imaging techniques. Efforts were also made by them to view the accuracy of these measurements in detection of stripe rust disease. In congruence with these studies, we have obtained significant correlation between NDVI measurements and stripe rust severity (Table.2) further confirming the facts. This supports our hypothesis that hand held remote sensing based studies can be used for quantifying disease severity of stripe rust in wheat.

Positive correlation (Figure. 3) was obtained for AUDPC vs CT (r = 0.46) showing that canopy temperature rises when leaves that contributes to transpirational cooling is severely affected by disease. Canopy temperature acts as an indirect indicator of the transpiration at whole plant level

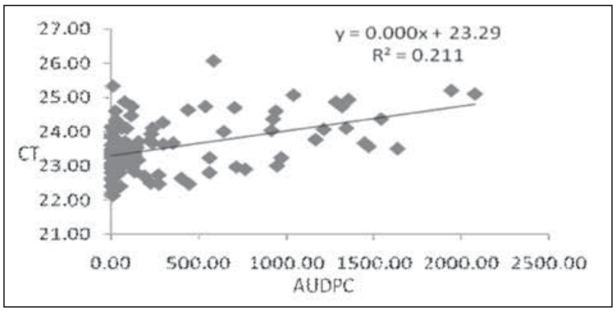

Fig 3. Plot of AUDPC as a function of Canopy temperature

and water status of plant (Araus *et al.*, 2003) and thus can act as an indicator of plant health. Thermal images can capture the heat emitted by plants under stress (Ortiz *et al.*, 2011). Earlier studies with canopy temperature measurement through infrared techniques were largely focused on the relationship between canopy temperature and drought tolerance (Reynolds *et al.*, 2001; Leinonen & Jones, 2004; Gonzalez-Dugo *et al.*, 2005; Mohammadi *et al.*, 2012). However, the present study illustrates scientific evidence to extend these techniques for rapid and precise quantification of disease severity which would further help in assessment of minor genetic variations in disease resistance.

Results obtained in the present study, confirms the fact that these spectral canopy indices can be used to quantify damages due to stripe rust. However, handheld instrument for measurement makes this study unique to already reported results where spatial techniques were used. In comparison to the earlier reports on use of spacial techniques to study plant health , where they had to use large area for their study (Bravo *et al.*, 2003; Franke & Menz, 2007), the present study depicts that handheld instrument can effectively be used for small areas. Among the three measurement indices (Table 2), maximum correlation with disease severity was observed between AUDPC and NDVI which was followed by chlorophyll content and canopy temperature, respectively. This can be attributed to the fact that Green Seeker sensor used for NDVI takes several readings by scanning the crop canopy and delivers an average value which apparently gives better representative information. However, as only a limited number of plants are used to take the measurements of chlorophyll content index, this may not be the best suited method for representation of whole plot. The fact that NDVI can effectively reflect the health and chlorophyll content of the plants was further strengthened by the fact that on correlating the measurement indices NDVI and CCI, we obtained significant relation ($p<0.001$) of 43% among them. Thus, physiological status as indicated by chlorophyll can also be explained by NDVI.

The present study, not only provides new methodology for screening disease in terms of precision but also can be used as a new approach to save time and money. Manual estimation of plant heath requires lots of labour and monitory support. To achieve the level of precision provided by NDVI , the man power required would be of estimated three times to that required if readings are taken manually. It is estimated that through present technique approximately 1000/- rupees (~16$) and 2 days of highly skilled technician can be saved to screen 100 samples in single replication (Table 3).

Table 3. Approximate cost and time saved in screening 100 genotypes in single replication

Vegetation Index	Approximate cost saved in screening 100 genotypes in rupees (equvivalent in US$))	Approximate time saved to screen 100 genotypes
NDVI	1000/- (US $16/-)	2.0 man days
CT	500/- (US $8/-)	0.5 man days
CCI	1000/- (US $16/-)	2.0 man days

Similar is the case for canopy temperature, where manual recording through use of thermometers can be very tedious and time consuming which can only be done through skilled persons. Therfore, there could be a difference of rupees 500/- (~8$) and 0.5 skilled mandays in generating the same quality of canopy temperature data as generated by canopy temperature instrument for 100 genotypes each. Chlorophyll content in a plant indicates health of the photosynthetic machinery. To estimate the content of chlorophyll in plants using various chlorophyll extraction methods, a large amount of time in the term of skilled manpower is required. It is estimated in the present investigation that, using chlorophyll meter can save around one day and 1000/- rupees (~16$) per 100 genotypes.

In conclusion**,** hypersensitive major gene resistance in rusts although have proven more effective but they get entrapped into a boom and bust cycle. Therefore, wheat improvement programmes now are repeatedly using non-hypersensitive, additive (quantitative) resistance which shall provide durable resistance. A sizeable correlation with AUDPC was obtained for all measurement indices, however, NDVI was found to be maximally correlated with r^2value of 0.658 and holds the maximum potential to discriminate between small variations enabling genetic studies and enhancement of disease resistance. Also, there is a scope for further studies to utilize these techniques for high through put precise phenotyping which can save time and money breeding disease resistance.

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