Identifying root rot (*Fusarium culmorum*) tolerant genotypes in wheat by using the RGB image-based vegetation indices

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Summary

Fusarium culmorum, also known as the crown rot fungus, causes significant damage to a wide variety of host plants worldwide. It is a difficult process to assess the susceptibility/tolerance of the genotypes. Digital image analyses are not only used to assess biomass and leaf area index, and for determining the chlorophyll content, radiation use efficiency, assessing drought, and in-season yield estimation but also used to determine and monitor mainly the foliar diseases in different crops. As the soil borne diseases affect the biomass and yield of the crop, RGB based vegetation indexes may be useful to assess the resistance/tolerance to soil borne diseases. The inoculated and non-inoculated trials were planted next to each other in Konya, Turkey in 2018-19 growing season. The photos were taken at different dates during the growing season and the yields were measured in both conditions. In average, the overall yield decrease was 7.9 % in the inoculated area compared to the non-inoculated area. The percent yield decreases were 12.8, 3.9, 7.4, 11.2, and 13.1 percent in resistance classes of 1, 2, 3, 4, and 5, respectively. The highest decrease was observed in the most susceptible genotypes with 13.1 %, the lowest decrease was in score 2 with 3.9 %. Correlations between RGB based image indexes and yield were similar to the Normalized Difference Vegetation Index (NDVI) reading, however the pattern in the Crop Senescence Index (CSI) was different from the others. Initial results indicates that RGB based vegetation indexes may have potential to assess the resistance/tolerance of the genotypes, however further detailed studies needed to assess the potential of it.

Introduction

Fusarium culmorum, also known as the crown rot fungus, is a soil-borne pathogen that causes significant damage to a wide variety of host plants (Nelson et al. 1993; El-Kazzaz et al. 2008). They can cause prominent disease symptoms in cereals such as crown rot, head blight, scab, and stalk rot. These symptoms have a negative impact on wheat production areas, resulting in yield losses worth billions of dollars (Windels 2000). *F. culmorum* is found in soil and plant matrices, including roots, aerial tissues, and organic substrates (Aoki et al. 2003). Cereal root rot symptoms include discoloration, necrosis, or rotting of the roots, sub crown internode, crown, and/or stem base (Stack 1982). Symptoms of severe root rot include stunting and wilting. RGB sensors are known to be useful for monitoring disease infection severity (Sugiura et al. 2016; Tetila et al. 2017; Vergara-Diaz et al. 2015; Feng et al. 2022). For plant phenotyping, RGB images are generally used to calculate NDVI, height, biomass, yield, etc. (Xie and Yang 2020). High-resolution RGB images have been used for the first time for wilt disease (*Fusarium oxysporum* f. sp. *raphani*) detection in radish (Dang et al. 2020). The objective of this study was to reveal the potential of RGB based imaging on the detection of tolerant wheat genotypes to *F. culmorum*.

Methods

Seventy-one advanced lines, with two checks, which are repeated 5 times in each replication were planted in Konya, Turkey to determine the yield loss under root rot inoculated conditions. The entries were planted as inoculated with *F. culmorum* and non-inoculated conditions; the trials were in the same field next to each other. The *F. culmorum* isolate was obtained from an infected wheat plant. The spore suspension was prepared by adding sterilized distilled water to each Petri dish containing *F. culmorum* culture. Wheat bran was inoculated with spore suspension. The fungus-colonized wheat bran was dried at room temperature and used for field experiments to inoculate the seeds of genotypes before planting.

Field experiments were conducted at Bahri Dağdaş International Agricultural Research Institute in Konya, Turkey in the 2018-2019 growing season. For each line, 140 g of seeds were planted in 6 m^2 rows and infested with an addition of 20 g of fungus-colonized wheat bran. The experimental trial design was Alpha Lattice with two replications in both conditions. Common agronomic practices for wheat were applied after planting: planting was done in October, 40/40 Kg/ha P_2O_5/N fertilizer was applied during the planting and additional 40 Kg/ha nitrogen was applied in spring during the tillering stage of the crop. Weed control was done by herbicides, and harvest was done in mid-July. Crown rot resistance-susceptibility responses of the entries were determined in a controlled environment, and they were scored from 1 to 5 on the following scale: 1 = Resistant (R), 2 = Moderately resistant (MR), 3 = Moderately susceptible (MS), 4 = Susceptible (S), and 5 = Highly Susceptible (HS). Digital photos and NDVI readings were taken every around 15 days starting from 06 March until 03 June during the growing season of the wheat. Photos were taken with a Sony a6000 camera (https://www.youtube.com/watch?v=O4oXwxVZLDM) using a focal length of 16 mm and keeping a constant distance between the camera and plot about 80-100 cm. The RGB digital image-based vegetation index, green area per meter square (GAm^2), Greener Green Area per meter square (GGAm^2), Early Vigor (EV), and Crop Senescence Index (CSI) were calculated using equations from BreedPix open-source software. NDVI measurements were made by handheld greenseeker "The Trimble GreenSeeker handheld crop sensor" (https://agriculture.trimble.com/product/greenseeker-handheld-crop-sensor/) at the same dates with photographs taken.

Spectral reflectance indices (SRIs) have been developed based on simple mathematical formulae, such as ratios or differences between the reflectance at given wavelengths. Simple ratio (SR=NIR/VIS) while Normalized Difference Vegetation Index (NDVI); NDVI= [(NIR-VIS)/(NIR+VIS)] = (R840-R670)/(R840+R670). These calculations were used to assess biomass and leaf area index. SRIs have been used for determining the chlorophyll content, radiation use efficiency, assessing drought, and in-season yield estimation.

ANOVA analysis was done for yield and correlations between RGB-based vegetation indexes and yield were calculated in Excel software.

Results

The number of entries in Resistance/Susceptible groups were 9, 20, 40, 6, and 6 from 1 to 5, respectively (Table 1). While the average yield was 4766.8 Kg/ha in non-inoculated area it was 4390.6 Kg/ha in inoculated areas, in average, the overall yield decrease was 7.9 % in the inoculated area compared to the non-inoculated area. The percent yield decreases were 12.8, 3.9, 7.4, 11.2, and 13.1 percent in resistance classes of 1, 2, 3, 4, and 5, respectively. The highest decrease was observed in the most susceptible genotypes with 13.1 %, the lowest decrease was in score 2 with 3.9%.

Table 1. The number of genotypes in each resistance/susceptible class, yield, and yield decrease of them under the non-inoculated and inoculated area

Resistance Class	The number of entries in the class	Average yield of the group in the non-inoculated area (Kg/ha)	Average yield of the group in the inoculated area (Kg/ha)	Yield decrease in inoculated area (%)	Yield decrease (Kg/ha)
1	9	4870.8	4248.3	12.8	622.6
2	20	4704.1	4522.6	3.9	181.5
3	40	4638.2	4295.5	7.4	342.7
4	6	5726.3	5086.8	11.2	639.4
5	6	4717.5	4101.3	13.1	616.3
Average		4766.8	4390.6	7.9	376.2

Correlations between digital images, NDVI, and yield under inoculated conditions are very low, non-significant, in the early growth stages of the plants (Figure 1), however it increases in the later crop growth stages. The correlations between yield and EV, GAm^2, GGAm^2, and NDVI in the first five measurements were negative, then they were high and positive in the last measurements. The correlations between CSI and yield under inoculated conditions followed a different pattern from the others; although there were not any statistically significant correlations in the first 3 measurements, it tended to be negative in next two measurements and reached the highest positive correlation in the measurement made on April 23 with 0.18. Then, the highest negative correlation was observed in the last measurement made on June 03.

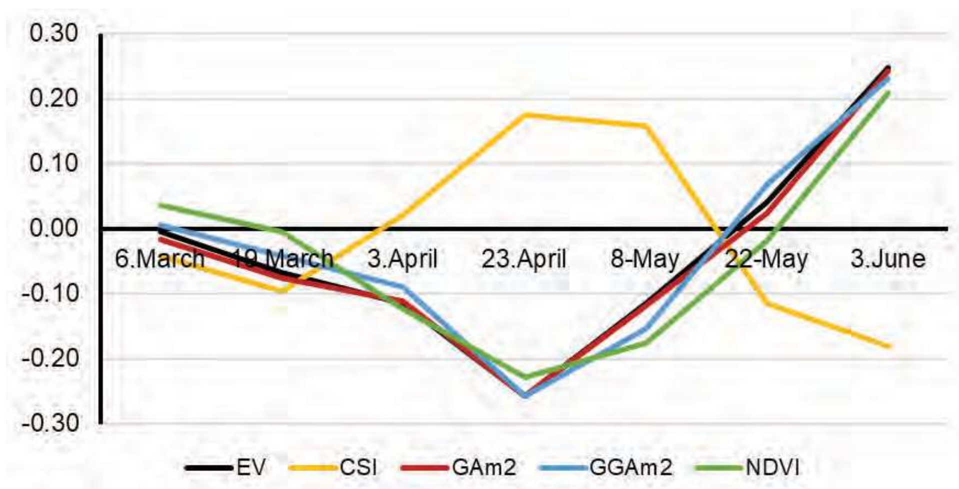

Figure 1. Correlations between yield and digital images under inoculated conditions. The X-axis represents the dates of measurements. The Y-axis represents the corresponding values for each vegetation index

Conclusion

RGB image-based vegetation indexes EV, GA, GGA and CSI could be used for estimation of potential grain yield at the early stage of crop growth, and RGB image-based vegetation indexes explained genetic variation in grain yield though correlations are very similar with NDVI. Initial results indicate that digital images and NDVI have the potential to assess the resistance/tolerance over the yield reduction of susceptible genotypes under inoculated conditions. However, more detailed studies are needed to confirm results and validation of the method's ability to identify clearly the resistant/tolerant genotypes. If a fast, cheap, repeatable, reliable and non-destructive screening method can be developed, higher number of genotypes can be screened in a shorter time to identify resistant/tolerant genotypes to RR/CR that could be used in breeding program. The RGB image-based vegetation indexes may have potential in that sense.

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