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EFFECTS OF THE INTERACTION OF FUSARIUM HEAD BLIGHT AND STRIPE RUST ON WHEAT YIELD PARAMETERS

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Fusarium head blight (FHB) caused mostly by *Fusarium graminearum* Schwabe and stripe rust caused by *Puccinia striiformis* are two most destructive diseases of wheat crop infecting ear and leaf respectively. Their independent effects on wheat yield are clear, but the effect of interaction between them is less reported. In the present study, the F_2 population derived from the cross combination L661 / L693 and PCA analysis was employed to estimate the influence of the interaction between FHB and stripe rust on yield parameters including GPS, GWS and TGW. The results showed that FHB has the larger effect on SGW and the smaller GPS as compared to stripe rust, and that the independent effects of both FHB and stripe rust on all yield parameters were significant at $p = 0.01$, but that of the interaction between them was more significant than both independent effects. In addition, comparing the difference in the effect on wheat yield between FHB and stripe rust indicated that the influence on wheat yield of FHB was underestimated in the past, possibly because many scientists paid more attention on the effects of FHB on quality rather than yield.
Keywords: Wheat diseases, source-sink relationship, kernel development, leaf disease, grain yield, *Pst* infection, principle component analysis.
Abbreviations: Fusarium head blight (FHB), Stripe (Yellow) rust (Sr), Principle component analysis (PCA), Grains per spike (GPS), Grain weight per spike (GWS), 1000-grain weight (TGW), Disease index (DI)

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the major crop in the world, contributing to about 20% of the human's caloric requirement. However, Fusarium head blight (FHB) caused mostly by *Fusarium graminearum* Schwabe (teleomorph Gibberella zeae (Schw.) Petch) and stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (Pst), occurring in the ear and leaf, respectively, are two destructive diseases causing a great yield loss (Yang *et al.,* 2016; Li *et al.,* 2015). In history, there were some FHB epidemics in wheat and barley (*Horderum vulgare* L.) in numerous wheat-growing regions and serious financial losses around the world (McMullen *et al.,* 1997; Nganje *et al.,* 2004).

In China, FHB has primarily happened within the center and lower valleys of the Yangtze River, and it is detailed that there were 7 extreme and 10 direct scourges of FHB from 1951 to 1990 within these areas (Yao and Lu, 2000). FHB moreover happened and ended up progressively serious within the southwest locale of China, in the recent years (Zhang *et al*., 2011). The FHB epidemic occurring in 2012 within the Yellow and Huai River valleys is the foremost genuine and the largest (Li *et al.,* 2017). A latest study proposed that FHB had a larger reduction within the 1000-grain weight and add up to grain weight per spike within the FHB vulnerable line as compared to the FHB resistance line (Yang *et al.,* 2016), which is explained in view that FHB influences kernel development (Del Ponte *et al.,* 2007). It is obvious that though the research about the effect of FHB on wheat yield loss is less than those of the mycotoxins accumulation, the independent effect of FHB on yield parameters had been elucidated.

Stripe rust, caused by *Puccinia striiformis*, is the most devastating leaf disease and a major productivity constraint for wheat across the world, and China is the largest region for wheat stripe rust, causing large yield losses, in the world (Luo *et al.,* 2005; Liu *et al.,* 2013; Huang *et al.,* 2014). As a leaf disease, stripe rust infection is closely related with leaf photosynthesis and senescence, which has been demonstrated by many of the expressed sequence tags induced by *Pst* and involved in those two processes (Mallard *et al.,* 2008; Wang *et al.,* 2009; Zhang *et al.,* 2013). Further studies suggested that the degree of stripe rust influencing grain yield depend on the disease response (Yang *et al.,* 2008) and the host growth stage at *Pst* infection (Murray *et al.,* 1994). In fact, stripe rust influences not only wheat grain yield, but also grain yield component by altering the distribution of assimilates among the various organs of the plant (Doodson *et al.,* 1965;

Siddique and Manners, 1971). Clearly, the independent effects of stripe rust on wheat yield are also well known.

The perfect development conditions for stripe rust are temperatures of between 8-13°C for spore germination and entrance, and 12-15°C for more advancement and with free water. This makes it a predominant infection in most wheat developing regions of china due to their cool and mild climate (Roelfs *et al*., 1992; Stubbs, 1985, 1988; McIntosh and Brown, 1997; Boshoff *et al*., 2002; Line, 2002; Wellings and McIntosh, 1990; Wellings *et al*., 2003; Yahyaoui *et al*., 2002). Moreover, stripe (yellow) rust proved to be the most damaging disease of wheat in china (Li *et al.*, 1984; Stubbs, 1988; Li and Zeng, 2000; Li and Liu, 1957; Lu *et al*., 1958; Li 1980; Wan *et al*., 2004; Zeng, 1963a,b, 1979). Stripe rust affects plant photosynthetic source, which in turn, causes 30-40% yield losses in wheat (Singh and Singh, 2002).

The tasks and biological functions of various plant organs exhibit wide variation. For example, the foremost vital capacities of the leaf as a "source" tissue is to produce energy-rich carbohydrate molecule through photosynthesis whereas the foremost vital part of the emerging seed as a "sink" is to store that carbohydrate molecule (Sturm and Tang, 1999). As far as wheat diseases are concerned, FHB pathogen mainly attacks spike tissues and therefore affects seed increase (Del Ponte *et al.,* 2007); *Pst* mainly infects leaf tissues and thus affects both seed formation and advance (Doodson *et al.,* 1965; Siddique and Manners, 1971). There is a dynamic balance between photosynthetic sources and nonphotosynthetic sinks (Luo *et al.,* 2009, 2013), and this balance could be changed with the manifest alterations in photosynthesis-related parameters by the direction of cellular signaling homeostasis when plants were attacked by pathogens (Rodriguez-Brljevich *et al.,* 2010; Wituszynska *et al.,* 2013). Leaf photosynthesis can be regulated by manipulating source sink relationship (Damatta *et al.,* 2008; Quentin *et al.,* 2013).

The photosynthetic activity of source and storage capacity of sink can greatly affect grain yield (Wang *et al*., 1997; Emam and Seghatoleslami, 2005). During grain filling stage, final grain mass is affected by the source ability to provide assimilates. In contrast, it has also been observed that sink limitation affects grain yield in most conditions (Borrás *et al*., 2004; Serrago and Miralles, 2014; Jenner, 1979; Slafer and Andrade, 1991; Savin and Slafer, 1991; Slafer and Savin, 1994; Kruk *et al*., 1997; Miralles and Slafer, 1995). A proficient transport of assimilates is required for high yield and improved grain filling. Source-sink relationship in plants affects the dry matter production in wheat.

The aim of current research is to evaluate the independent as well as interaction effects of FHB and SR on plant yield parameters and to assess the source-sink relationship in wheat plant by estimating the correlations between different yield parameters under four different sets of disease stress conditions. The amount of variation being contributed by the individual traits has also been estimated.

MATERIALS AND METHODS

Plant material and experimental design: The wheat line L693 with both FHB and stripe rustresistance and the susceptible L661 to both FHB and stripe rust (Zhang et al., 2011; Liu et al., 2015; Li et al., 2016), and the total of 471 F2 plants derived from the cross 'L661/L693" were used to determine the interaction between FHB and stripe rust because L693 and L661 are sister lines with high similar genetic backgrounds (Huang et al., 2014; Li, 2015). All materials were planted in the field at Yaan (lat. 29°59'N, long. 102°58'E) in 2011. which is a famous 'rainy city' in the world and a temperate rainy environment, and it was reported that the yearly average temperature is 15 to 17°C and the yearly average precipitation is 1,520 mm with about 240 rainy days each year

(Luo et al., 2009), which is helpful for wheat disease occurrence and prevalence. The parent lines field trials experiments were adopted with a randomized completeblock design with three replication and the randomly segregating F2 plants were planted within a replication.

***Resistance evaluation*:** In the present research, a macroconidial suspension having 200 macro conidia/ml was showered on the spikes of around 200 plants. In order to screen for disease resistance at anthesis stage, these plants were arbitrarily selected from each plot. Conidial suspensions were prepared from a single spore-derivative segregate of *F. graminearum* No. 4 which was obtained on request from Professor Zhengqiang Ma at Nanjing Agricultural University, Nanjing, Jiangsu Province, China. The *F. graminearum* conidial suspensions were prepared by placing *F. graminearum* conidia into V_8 juice agar and were developed at room temperature for 1 to 2 weeks. Another, falcate conidia poured from the plates were exchanged into fluid mung bean medium and refined for 3 days at 25°C by shaking at 150 rpm. At last, the conidial suspension was centrifuged at 8,000 rpm for 10 min after sieving by Miracloth. It was then fixed in an ice bucket in order to transport to the field, and finally utilized for inoculation in 5 h. Heads from the main stem were haphazardly checked to assess FHB resistance.

***Statistical analysis*:** Significant contrasts in the mean disease severity and yield traits of arbitrarily chosen plants from three replications between the resistant, susceptible and among the four sets of disease conditions of two wheat lines (L693 and L661) were determined using an autonomous samples t test with IBM SPSS Statistics 19 software (SPSS Inc., Chicago, IL). Furthermore, noteworthy variations in the mean yield traits between both the resistant, susceptible and among all the four sets of disease conditions were also determined by the same program.

***Principle component analysis (PCA)*:** R software was utilized to conduct principle component analysis in order to partition the total variation present in the variables and the traits under observation into its principle components. Multivariate analysis was done to estimate the correlations between all the traits, density distribution of the sample data, regression line for all the traits and the box plots for all the traits under four different sets of disease conditions. Scree plot was made to demonstrate the percentage of explained variance. Graph of variables has been constructed to show the contribution of all the traits. PCA biplots were constructed to estimate the important associations between the different traits under all the four sets of disease conditions.

RESULTS

Multivariate combined analysis in Figure 1 demonstrates the correlation between all the traits, the density distribution of the sample data, regression line for the traits and the box plots for all the traits under four different types of disease conditions. The last column in Figure 1 shows the box plots for each variable under four different sets of disease conditions. Spike grain weight and grains per spike exhibit the maximum value when the plant is resistant to both the diseases and also when it is FHBr-SRs while disease index appeared to be the maximum when the plant was susceptible to both the diseases and also during the phase of FHBs-SRr.

In the correlogram (Fig. 1), grains per spike showed a strong positive correlation of 0.89 with spike grain weight and the later showed strong positive correlation of 0.739 with thousand grain weight in all the four types of disease conditions (0.918, 0.89, 0.798 and 0.8, respectively). However, a strong negative correlation of -0.646 has been found between disease index and thousand grain weight in case of FHBs-SRs and similar correlation of -0.586 has been observed in case of FHBs-SRr except with the additional correlation of -0.524 with spike grain weight.

The scree plot in Figure 2 demonstrates the percentage of explained variances or eigenvalues by all the traits of interest. In this data, approximately 53% explained variances are due to grains per spike, 17% by spike grain weight, 8.4% by thousand grain weight while only 1.4% by the disease index. This shows that 70% of the explained variances are within the first two dimensions or principle components.

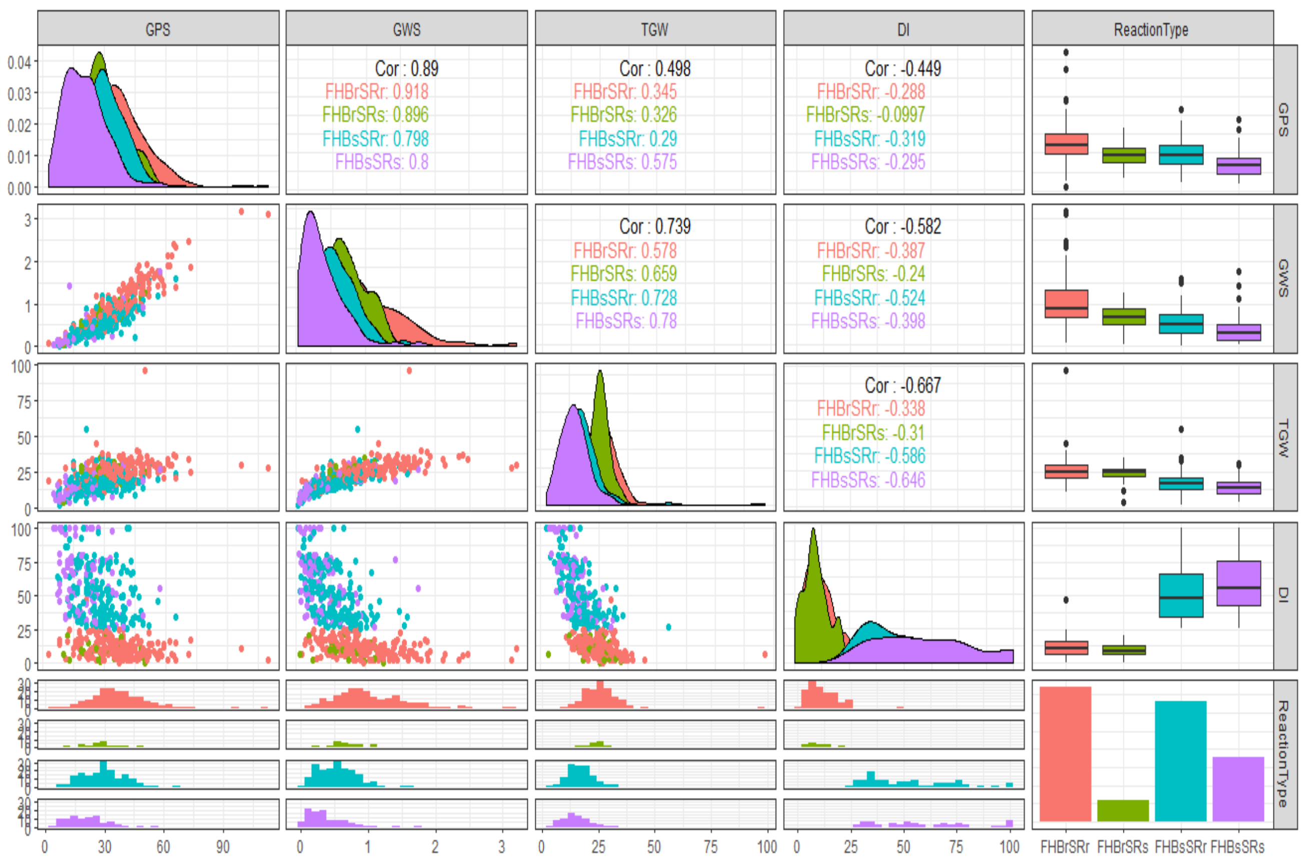

Figure 1. Multivariate combined figure showing correlation between all the traits, the density distribution of the sample data, regression line for the traits and the box plots for all the traits under four different types of disease conditions.

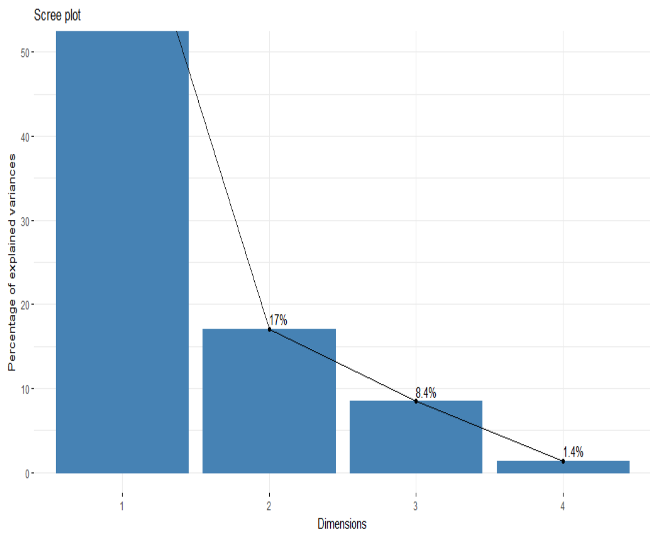

Figure 2. Scree plot (Eigenvalues or variances explained).

Figure 3 shows the contribution of the four traits. Grains per spike and spike grain weight lie in the same dimension contributing their maximum contribution towards the total variation. Both are positively related to each other proving themselves the best traits to be studied in further studies. Disease index is also contributing towards variation but in the opposite direction.

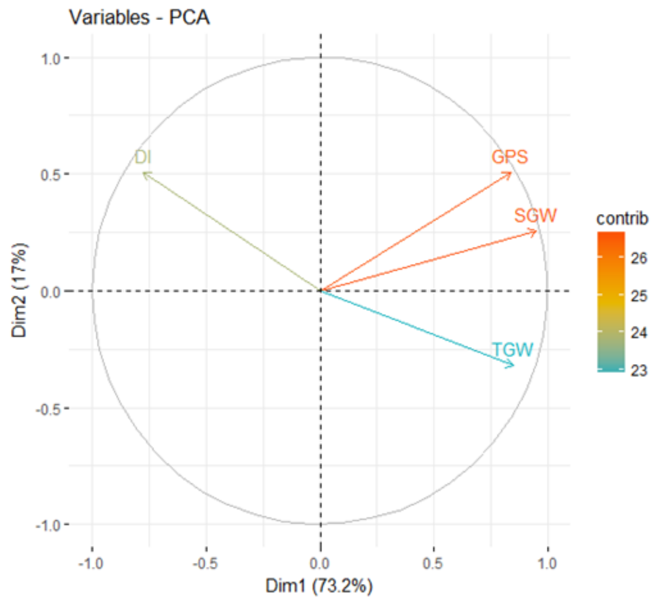

Figure 3. Variables PCA showing contribution of traits towards total variation.

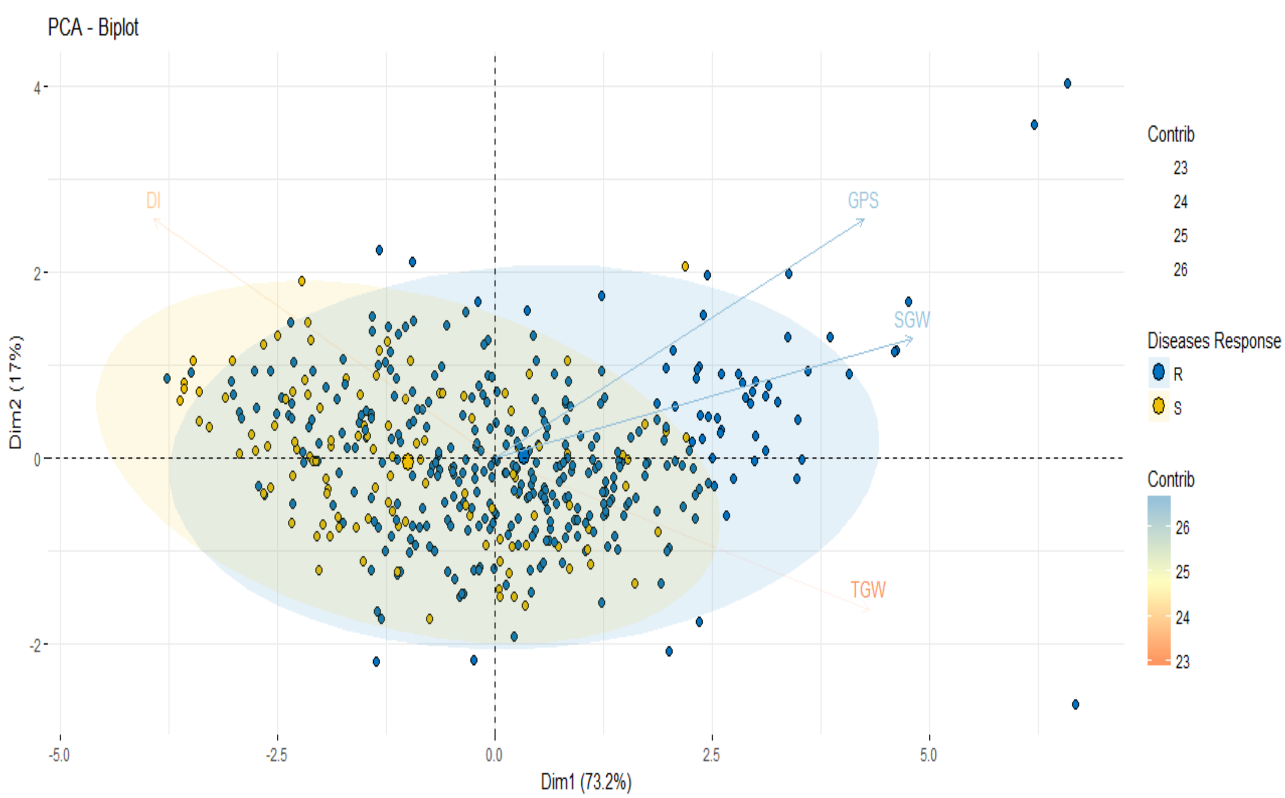

Figure 4. PCA biplot showing disease eclipses for the traits.

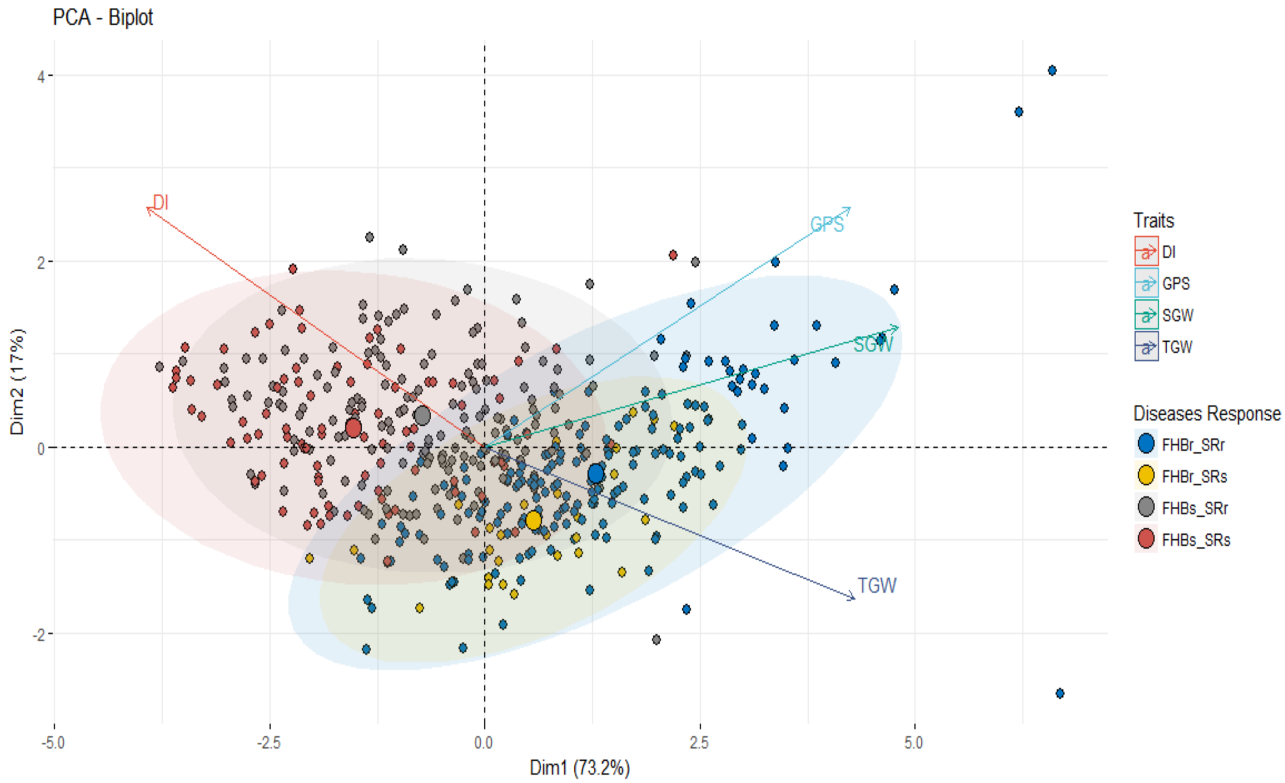

Figure 5. PCA biplot showing disease eclipses for each of the four disease conditions and each of the four traits.

The PCA-biplot in Figure 4 shows the direct relationship of grains per spike and spike grain weight with plant resistance mechanism while disease index is associated with susceptibility.

The PCA-biplot for all the traits under the four sets of disease conditions in Figure 5 depicts that grains per spike and spike grain weight lie positively close to each other and are associated with FHBr-SRr, thousand grain weight exhibit its association with FHBr-SRs while disease index lie in the opposite direction associated with FHBs-SRr and FHBs-SRs.

Table 1. The effects of interaction between FHB and SR on wheat yield parameters GPS, GWPS, and KGW.

Group	GPS	GWPS	KGW
FHB (R) SR (R)	37.1±1.07a	1.0±0.04a	25.9±0.59a
FHB (R) SR (S)	28.9±1.90b	0.7±0.05b	23.8±1.11a
FHB (S) SR (R)	28.5±0.82b	0.6±0.02c	17.3±0.52b
FHB (S) SR (S)	20.6±1.10c	0.4±0.03d	14.6±0.61c

FHB, Fusarium head blight; SR, stripe rust; GPS, grain per spike; GWPS, grain weight per spike; KGW, 1000-grain weight; R represents resistance; S represents susceptibility; Lower cases symbolize significant at the probability level of 0.05 (LSD); upper cases symbolize significant at the probability level of 0.01 (LSD).

Table 2. The effect of different FHB and SR resistance traits on wheat yield parameters GPS, GWPS, and KGW.

Disease	Phenotype	GPS	GWPS	KGW
FHB	R	36.0±0.98**	1.0±0.03**	25.6±0.53**
	S	25.8±0.70	0.5±0.02	17.1±0.80
SR	R	33.1±0.72**	0.8±0.03**	21.8±0.46**
	S	22.7±0.98	0.5±0.03	18.4±1.62

FHB, Fusarium head blight; SR, stripe rust; GPS, grain per spike; GWPS, grain weight per spike; KGW, 1000-grain weight; ** Significant at the 0.01 probability level; R represents resistance; S represents susceptibility.

Significant differences have been observed among the means of various wheat yield parameters under the different conditions of disease resistance and susceptibility. By comparing the combined effects of both the diseases, FHB and SR on the means of wheat yield parameters, it can be shown that FHB is affecting GPS, GWPS and KGW more harmfully than SR (Table 1). As the mean values of three yield parameters are most affected in both the conditions when the plant is susceptible to both the diseases or is susceptible only to FHB. However, the measurement of individual effects of both the diseases on wheat yield demonstrates the more harmful effects of SR on plant yield parameters as they all have been most affected when the plant was SR susceptible (Table 2).

DISCUSSION

The results showed that FHB has the larger effect on SGW and the smaller GPS have been observed as compared to stripe rust, and that the independent effects of both FHB and stripe rust on all yield parameters were significant at p = 0.01, but that of the interaction between them was more significant than both independent effects. In addition, comparing the difference in the effect on wheat yield between FHB and stripe rust indicated that the influence on wheat yield of FHB was underestimated in the past, possibly because many scientists paid more attention on the effects of FHB on quality rather than yield.

Different studies have been conducted to understand the plant source-sink mechanism in order to improve the design of plant disease management. Most of these studies involved defoliation of flag leaf or all leaves and the removal of spikelets for estimation of the effects of source or sink limitation on grain yield. Source or sink limitation may arise in various crops during various stages of development and physiological maturity. However, in cereals, grain filling proved to be the most important stage for the transport and supply of assimilates which are then stored into the grain structure for its development. In case of wheat, the crop is mostly sink limited as mentioned in the literature (Borrás *et al*., 2004; Jenner, 1979; Miralles and Slafer, 1995; Richards, 1996; Kruk *et al*., 1997; Savin and Slafer, 1991; Slafer and Savin, 1994; Serrago and Miralles, 2014; Slafer and Andrade, 1991). On the contrary, major source limitations have been observed in the warmer climates as there is an early senescence of green parts in these areas (Fisher, 1983). The novelty of present research is the understanding of source sink limitation and its effects on grain yield components by affecting both source and sink through disease i.e., stripe rust and FHB respectively.

The prime objective of the current research is to characterize the effects of *Fusarium graminarium* and *Puccinia striiformis* infection on wheat grain yield parameters under different combination of plant disease conditions in terms of source-sink relationship. We examined some important yield traits along with disease index. The results demonstrated that the yield traits showed a significant decrease when the plant was susceptible to both the diseases, i.e., FHBs-SRs while a significant increase in disease index has been observed in this condition as compared to the condition when the plant was resistant to both the diseases, i.e., FHBr-SRr (Fig. 3). However, FHB invasion had a more negative effect on yield traits than the stripe rust as the yield traits showed a significant decrease and a significant increase in the disease index has been shown when the plant was FHBs-SRr as compared to FHBr-SRs (Fig. 3). The above results were further affirmed from the multivariate analysis in Fig. 5. Correlation studies further confirmed that FHBs-SRr plants showed same negative results of increasing disease index on the yield parameters as in FHBs-SRs plants (Fig. 5).

In terms of source-sink relationship in the present studies, FHB infection would be more affecting the spikes, i.e., photosynthetic sink of wheat plant while Plant leaves being the photosynthetic organ/source would be most affected by stripe rust. From the above results, it has been clearly revealed that the plant yield in this study appeared to be more sink-limited than source-limited because FHB infection destroyed plant yield more than the stripe rust infection. The disease indices remained almost the same when the plant was resistant to either both the diseases or to FHB while a significant increase in the disease indices has been observed when the plant was either susceptible to both the diseases or to FHB. The findings of Evans and Rawson (1970) are best to further describe the sink restraint to plant grain yield who considered spike and flag leaf blade photosynthesis necessary to fulfill the grain requirements throughout the grain filling stage.

Grains per spike and grain weight per spike appeared to be the most important trait to be considered for further studies because of their maximum contribution towards variation as well as the strongest association with plant resistance mechanism. Disease index also presented itself as the most suitable trait to link plant source-sink mechanism with FHB infection. The development of highly segregating F_2 population can further benefit future breeding programs including QTL analysis, marker assisted selection.

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