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CONTROL MEASURES OF ROOT ROT FUNGAL PATHOGENS BY PRODUCING RESISTANCE AGAINST TWELVE WHEAT CULTIVARS UNDER GREENHOUSE IN NEBRASKA STATE OF USA

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Abstract

Susceptibility or resistance against three strains of root rot pathogens of twelve wheat cultivars were analyzed in Nebraska State of USA. It was mainly focused on the screening of twelve wheat varieties against *Fusarium graminearum* strain W433 and two strains (G256 and W1624) of *Rhizoctonia solani*. These pathogens contributed to significant yield reduction. Agar plug inoculation and biological control agents culture suspension techniques were used for experimental trials. The disease severity was measured against three fungal strains by agar plug inoculation method (APIM). It was observed that wheat cultivars (WC) Freeman (2.00) and NW07505 (2.50) were moderately resistant while Overland (3.25), Overland FHB10 (3.00), Panhandle (3.75), Ruth (3.50), Mattern (3.75), RedHawk (3.00) and SY-Wolf (3.25) rated as susceptible against *Fusarium graminearum* strain W433. Overland FHB10 (1.75), Panhandle (2.00), NW07505 (3.00) showed moderate resistance and Freeman (4.00), Ruth (3.75), Mattern (4.25), McGill (4.00) rated as susceptible against *Rhizoctonia solani* strain G256. Similarly, *Rhizoctonia solani* strain W1624 showed less disease severity against Freeman (2.00), NW07505 (2.00), SY-Wolf (2.00) while more severity appeared against Overland (3.50), Robidoux (3.50), Ruth (4.00), Mattern (4.00). According to statistical evaluation, all artificially inoculated wheat cultivars (WC) with W433 strain showed almost similar shoot length (L) while fresh weight (FW) of shoot was slightly variable between treated and non-treated varieties. Similarly, root length, root FW and dry weight (DW) of wheat lines appeared significantly similar. It was estimated that all artificially inoculated WC indicated slightly reduced biomass in comparison to control after six week harvesting periods. Shoot length of five artificially inoculated WC with strain G256 as V_1 (Freeman), V_6 (Ruth), V_7 (Settlers), and V_9 (Mattern) showed less weight when compared with control. Wheat lines V_6 (Ruth), V_7 (Settlers), V_9 (Mattern) and V_{11} (RedHawk) also reduced shoot FW weight after pathogen inoculation. Dry weight of shoot was also reduced in seven varieties after inoculation of W1624 strain. It was noted that Rhizoctonia strain W1624 was more virulent to cause root rot disease and reduced more biomass of yield components in comparison to Rhizoctonia strain G256.

Key words: Management, Resistance, *Fusarium graminearum, Rhizoctonia solani,* Wheat, Pathogens.

Introduction

Triticum aestivum L. is the most important cereal crop after rice in the world. It is being the staple food for about one-third of the world population. Human beings eat wheat grains more as compared to other cereal crops. Role of world economy for wheat production is significant in terms of both food supply and cultivated lands. China is expected to be first in wheat production ranking in the world and ranged first in total food grain production (Foreign Agriculture Service, 1984; China Reconstructs, 1984). This crop ranked second most important crop in USA. Almost third fourths acreage of different states of USA (Nebraska, Kansas, Washington, Oklahoma, Montana, Texas, Dakota and Colorado) produce wheat crop. The Northwest is the principal white wheat producing area in the United States and a major supplier for both national and international markets. In 2008, soft white wheat accounted for 79% of total wheat production in Washington State. Actually, more than 46% of all United States white wheat comes from Washington alone (E.R.S., 2009).

Plant pathogens are a major yield limiting factors in cereal crops throughout the world. The most ubiquitous of all the pathogens are the soil borne organisms, which are present in all cereal growing regions. Plant-pathogen interactions are mediated by a complex network of molecular and cytological events that determine a range between susceptibility and resistance. Plants possess a range of active defense responses that contribute to resistance against a variety of pathogens. They respond to fungal pathogen attack by activating various defense responses that are associated with the accumulation of several factors like defense related enzymes and inhibitors that serve to prevent pathogen infection. The interaction between the pathogen and host plant induces some changes in cell metabolism, basically changes occur in the enzyme activities (Kini *et al*., 2000; Shivakumar *et al*., 2002; Babitha *et al*., 2004; Girish & Umesha, 2005).

Among the various factors responsible for low yield of wheat in USA, fungal diseases play a vital role. Like other crops, wheat suffers from a number of seed and soil borne diseases (Ahmed, 1994). The fungi *Rhizoctonia solani* and *Rhizoctonia oryzae* cause a root rotting disease of wheat. Rhizoctonia root rot is currently a major problem in the Pacific Northwest United States, Australia and parts of Europe (Pumphrey *et al*., 1987; Smiley *et al*., 1990; Mathieson & Rush, 1991). This root rot disease is caused by *Rhizoctonia solani* that belongs to primitive Basidiomycetes. The fungus grows saprophytically on dead plant remains, but it becomes vigorously parasitic when roots or other parts of a susceptible host penetrate the infested zone (Watkins, 1981, Neate, 1985; Rovira *et al*., 1986; Rovira *et al*., 1986; Pumphrey *et al*., 1987; Ogoshi *et al*., 1990; Pumphrey *et al*., 1987). *R. solani* is the most damaging at root zone temperatures below 15°C

(Smiley *et al*., 1989; Ogoshi *et al*., 1990). The pathogen causes localized patchy, stunted areas in the field and is sometimes referred to as bare patch or purple patch (Smiley *et al*., 1989; Carling & Kuninaga, 1990; Ogoshi *et al*., 1990).

However, in the last two decades increasing number of papers were published on yield losses (30% to 50%) caused by Rhizoctonia species in the main wheat cultivating areas of the world (Chen *et al*., 2008; Hamada *et al*., 2011; Guo *et al*., 2012). In Europe and North America winter wheat suffered mainly due to *R. solani* AG-8 strains (Hamada *et al*., 2011) and with the *R. cerealis* (Chen *et al*., 2008; Hamada *et al*., 2011; Guo *et al*., 2012), while in Australia and Turkey five different anastomosis groups of *R. solani* (Tunali *et al*., 2008) were identified. The disease is more severe in sandy soils, as the fungus can grow more rapidly (Gill *et al*., 2000), and the hyphae tend to colonize rhizoplane reducing the vitality of plant even without penetrating into tissues.

Several species of Fusarium are usually involved in the spread of same disease but the most common Fusarium species causing root rots are *Fusarium graminearum* and *F. culmorum*. These two pathogens can act synergistically with other Fusarium species in a disease complex that causes more severe root and crown rot diseases. Fusarium root rot disease is caused by a fungus *Fusarium graminearum* inflicting tremendous economic losses by reducing grain yield and quality of wheat and soybean crops. In affected plants, root tissues invading pathogens are destroyed and water with nutrient uptake is ceased (Mesterhazy *et al*., 2005).

There are no known resistant varieties of wheat and barley currently available to the grower, although some varieties are known to vary in tolerance to the pathogens (McDonald, & Rovir, 1985). There are no reports of true resistance against *F. graminearum* within cultivable species and there are only very few commercial agronomic cultivars partially resistant to this pathogen. The isolates of different *Fusarium spp.* differed largely in quantitative aggressiveness; they did not show significant qualitative differences in their virulence (Akinsanmi *et al*., 2006; Sip *et al*., 2008). However, wheat breeding for general Fusarium resistance may be possible in some cultivars/lines of wheat crop (Kosova *et al*., 2009). Few reports have been observed in the world that developed resistant varieties of wheat against root rot diseases through screening (Ahmed & Bakar, 1991; Mishra *et al*., 1992; Harlapur *et al*., 1993, Ahmed *et al*., 2009). But reports on the development of resistant or tolerant varieties against fungal diseases of wheat is scanty in the country.

So, it should be the first and foremost duty to find out an appropriate control measure of soil-borne diseases of wheat varieties. Various methods are available for the control of soil-borne diseases (root rot diseases) of wheat varieties. But, development of resistant varieties is one of the safe, cheapest and reliable method for the control of root rot diseases of wheat varieties. Hence, the present research work focused on the screening of root rot diseases caused by *F. graminearum* and *R. solani* and to find out any resistant or tolerant variety against these root rot diseases of wheat for future cultivation. Therefore, this investigation was undertaken to evaluate the pathogenic variability and resistance among isolates of *R. solani* and *F. graminearum* originating from different regions in Nebraska State of USA and their interactions with 12 wheat cultivars under greenhouse conditions (Fig. 1).

Materials and Methods

Collection of fungal isolates: One strain of *R. solani* and one strain of *F. graminearum* were obtained from the preserved stock of Dr. Tony Adesemoye Laboratory, Department of Plant Pathology, West Central Research and Extension Center, North Platte, USA, and isolated from infected seeds of susceptible wheat cultivars. These strains were sub-cultured on PDA-t medium under aseptic conditions in laminar flow hood. Then these strains incubated at room temperature (25-28ºC) for seven days in growth chamber for further inoculation on wheat seeds sowing in containers under greenhouse conditions.

Collection of wheat varieties: Seeds of 12 wheat varieties were collected for screening of soil-borne diseases from Agricultural Department of Nebraska, USA. Then, these seeds were surface sterilized and preserved in the Laboratory for further experimental trails under greenhouse conditions.

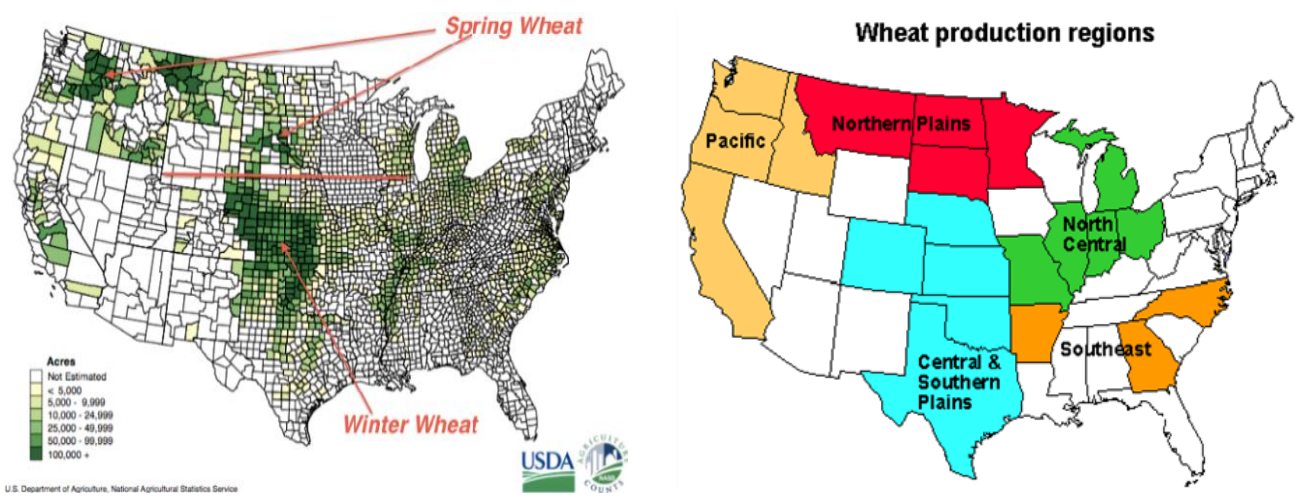


Fig. 1. Wheat production areas in Nebraska State of USA (Study Area).

Agar plug inoculation method: In the current technique, soil (culture medium) was put in each container under greenhouse conditions. Then a hole in the center of each container was dug up. After that seeds of each variety were sown in each hole. Thus, 24 seeds were taken from each variety. At the same time two holes were dug up in each container and inoculated two plugs of one pathogen in each container. Similar procedure was repeated for each container. The experiment was arranged as 12 X 3 factorials with 8 replicates using containers as pots (total of 288 containers). Two destructive samplings have done as that 4 replicates at 3 weeks after inoculation and the remaining 4 replicates at 6 weeks after inoculation. In this way, out of 288 containers, 192 containers were inoculated with two pathogens. So, 96 containers were inoculated with *F. graminearum*, 96 containers inoculated with *R. solani* and 96 containers did not inoculate (control). After that these all containers were irrigated according to given time frame (15 minutes twice a day). The samples collected after 21 days and at 42 days (two times). First observation of diseases has been done after 3 weeks and second observation has been completed after 6 weeks. These observations have been rated the seedlings for exploration of the pathogenicity (susceptibility). Other characters (root fresh & dry weight, height of plant, germination rate, length of longest root, shoot length, leaf size and leaf colour) have also been measured. Incidence and severity of diseases have been quantified against each variety for analysis of susceptibility or resistance. Then we concluded that which variety is better for future sowing in the study area (Dhingra & Sinclair, 1995; Michereff *et al*., 2008).

Statistical analysis of the data: The experimental design of the present study was a randomized complete block with three replicates. Analysis of variance (ANOVA) of the data and their correlations were performed with SAS Statistical Package. The least significant difference (LSD) was used to compare the means of isolate and cultivars.

Results

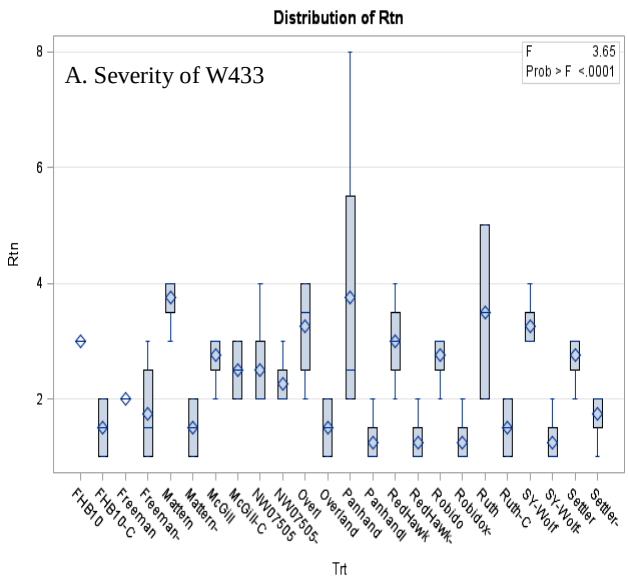
In the recent study, we analyzed susceptibility or resistance of twelve wheat cultivars (WC) under greenhouse conditions. These wheat varieties were commonly cultivated in Nebraska State of USA. Responsis of root rot causing pathogens of wheat cultivars against each group of wheat variety was

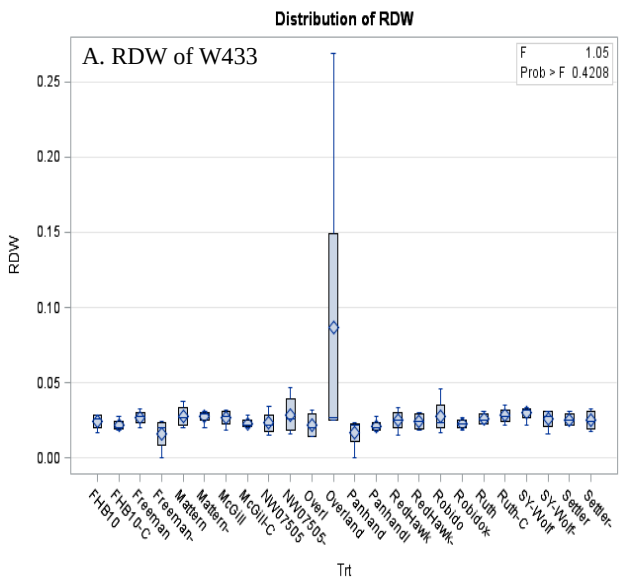
Response of wheat cultivars against root rot pathogens: The disease severity was measured against three fungal strains based on 1-8 rating scale as indicated in Table 1. It was observed that Freeman (2.00) and NW07505 (2.50) WC are rated as moderately resistant while Overland (3.25), Overland FHB10 (3.00), Panhandle (3.75), Ruth (3.50), Mattern (3.75), RedHawk (3.00) and SY-Wolf (3.25) rated as susceptible against *F. graminearum* strain W433. On the other hand, Overland FHB10 (1.75), Panhandle (2.00), NW07505 (3.00) showed moderately resistance and Freeman (4.00), Ruth (3.75), Mattern (4.25), McGill (4.00) rated as susceptible against *R. solani* strain G256. Similarly, *R. solani* strain W1624 showed less disease severity against Freeman (2.00), NW07505 (2.00), SY-Wolf (2.00) while more severity appeared against Overland (3.50), Robidoux (3.50), Ruth (4.00) and Mattern (4.00). This rating was assessed after comparison of inoculated and control samples. Statistically, it was observed that some cultivars showed variations. Different letters showed significant difference between values (Table 1). Further detailed statistical analysis of three strains of fungal pathogens of root rot diseases of wheat crop were explored and documented (Fig. 2A, B, C). Almost similar scoring was assessed after six weeks analysis. Therefore, severity results calculated after six weeks did not mention in the present research paper.

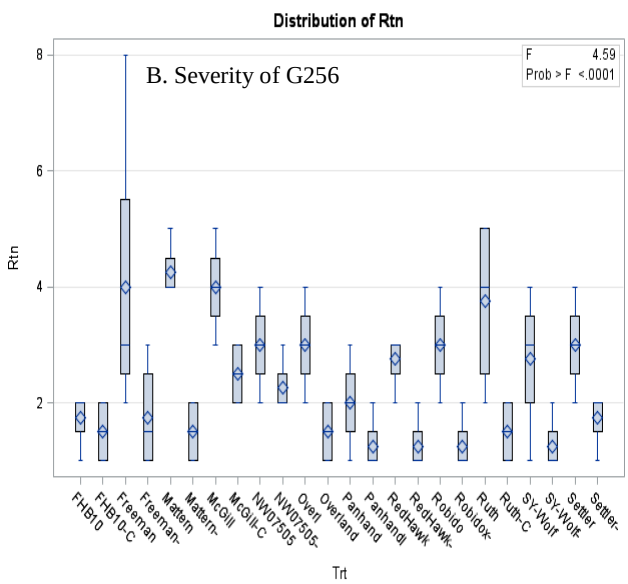
Table 1. Rating of 12 wheat lines inoculated with three fungal pathogenic strains W433, G256 and W1624 under greenhouse conditions after three weeks.

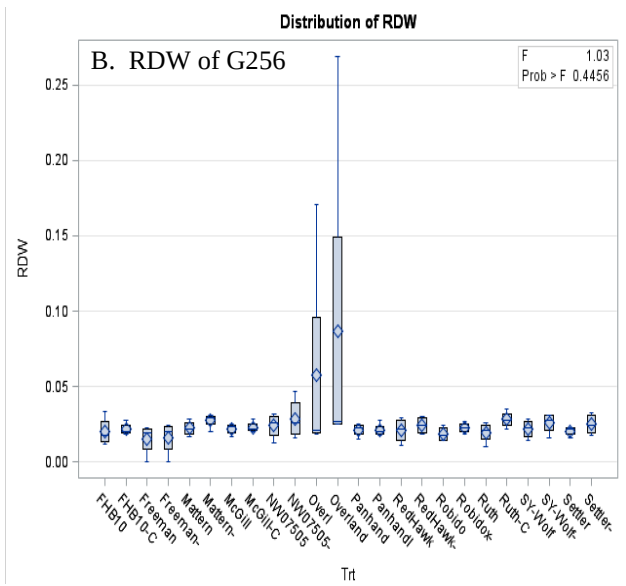
Wheat Lines	Fungal strains severity (1-8 rating scale)					
	W433	Control	G256	Control	W1624	Control
Freeman	2.00cdef	1.75def	4.00ab	1.75def	2.00 abc	1.50bc
Overland	3.25abc	1.50ef	3.00abcd	1.50ef	3.50 ab	1.25c
Overland FHB10	3.00abcd	1.50ef	1.75def	1.50ef	3.00 abc	2.00abc
Panhandle	3.75a	1.25f	2.00def	1.25f	2.25 abc	1.00c
Robidoux	2.75abcde	1.25f	3.00abcd	1.25f	3.50 ab	1.50bc
Ruth (NE10589)	3.50ab	1.50ef	3.75abc	1.50ef	4.00 a	1.25c
Settlers CL	2.75abcde	1.75def	3.00abcd	1.75def	2.50 abc	1.25c
NW07505	2.50abcdef	2.25bcdef	3.00abcd	2.25def	2.00 abc	1.50bc
Mattern W4265	3.75a	1.50ef	4.25a	1.50ef	4.00 a	1.50bc
McGill	2.75abcde	2.50abcdef	4.00ab	2.50cdef	2.00 abc	1.00c
RedHawk	3.00abcd	1.25f	2.75bcde	1.25f	3.50 ab	1.00c
SY-Wolf	3.25abc	1.25f	2.75bcde	1.25f	2.00 abc	3.50ab
LSD (0.05%)	**1.26**		**1.29**		**2.03**	

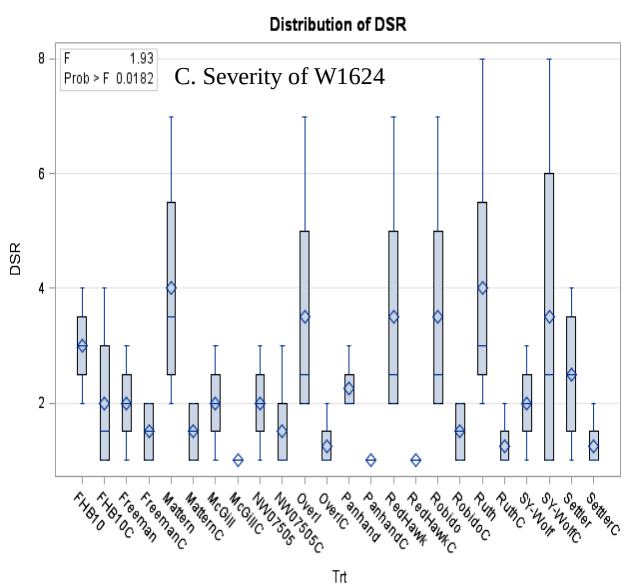
Key: W433 = *Fusarium graminearum*, G256 = *Rhizoctonia solani*, W1624 = *Rhizoctonia solani*











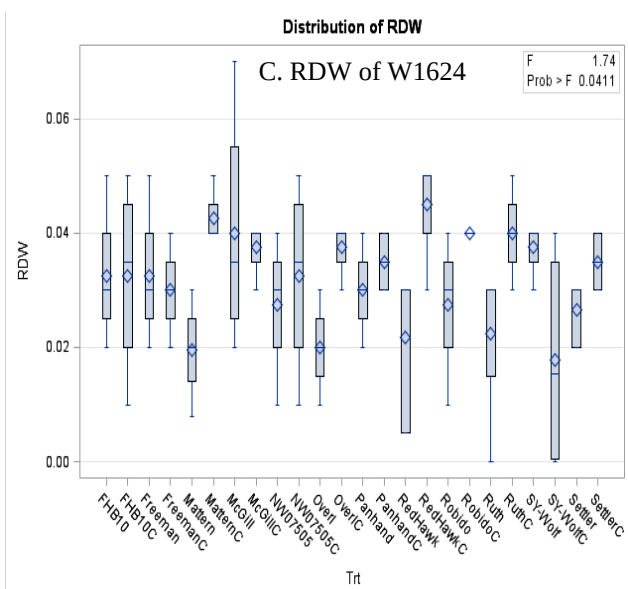


Fig. 2A, B, C. Disease severity of root rot diseases caused by Fusarium strain W433 and Rhizoctonia strain G256 and W1624 of 12 wheat cultivars.

Fig. 3A, B, C. Box plot showing statistical assessment of root dry weight of inoculated and non-inoculated experimental trails of 12 wheat varieties.

Table 2. Response to yield components of 12 wheat lines after inoculation of *F. graminearum* strain W433 under greenhouse conditions after three weeks.

WV	Shoot system						Root system						Leaves per plant	
	L (cm)		FW(g)		DW(g)		L (cm)		FW(g)		DW(g)			
	I	NI	I		I	NI	I	NI	I	NI	I	NI	I	NI
V_1	18.38a	14.30a	0.10abc	0.05c	0.05abc	0.03c	13.62a	14.88a	0.03b	0.02b	0.03b	0.02b	3.5abc	3.0c
V_2	15.75a	16.88a	0.10abc	0.09abc	0.06a	0.05abc	13.50a	13.78a	0.02b	0.03b	0.02b	0.09a	3.8abc	3.5abc
V_3	17.83a	18.25a	0.12abc	0.13ab	0.05abc	0.06a	13.75a	14.55a	0.03b	0.02b	0.02b	0.02b	3.8abc	4.5ab
V_4	15.13a	19.13a	0.12abc	0.11abc	0.04abc	0.05abc	10.55b	14.38a	0.02b	0.05a	0.02b	0.02b	4.0abc	4.8a
V_5	18.13a	19.38a	0.11abc	0.11abc	0.04abc	0.05a	14.00a	14.13a	0.02b	0.02b	0.03b	0.02b	3.5abc	4.5ab
V_6	16.38a	18.28a	0.14a	0.11abc	0.06a	0.05a	14.95a	14.15a	0.03b	0.03b	0.03b	0.03b	4.0abc	3.8abc
V_7	17.25a	19.75a	0.10abc	0.11abc	0.05abc	0.05ab	14.83a	13.95a	0.03b	0.03b	0.03b	0.03b	3.5abc	4.0abc
V_8	17.30a	15.55a	0.10abc	0.08abc	0.05abc	0.03bc	14.13a	14.63a	0.02b	0.02b	0.02b	0.03b	4.0abc	3.5abc
V_9	14.95a	17.63a	0.08abc	0.10abc	0.04abc	0.05abc	13.93a	14.20a	0.03b	0.03b	0.03b	0.03b	3.3bc	3.3bc
V_{10}	19.50a	14.13a	0.12abc	0.06bc	0.05abc	0.04abc	13.38a	14.43a	0.03b	0.02b	0.03b	0.02b	3.5abc	3.3bc
V_{11}	17.38a	19.50a	0.13ab	0.14a	0.05abc	0.05abc	14.60a	14.08a	0.03b	0.03b	0.03b	0.03b	3.5abc	3.3bc
V_{12}	17.83a	16.13a	0.11abc	0.10abc	0.05abc	0.04abc	14.10a	13.38a	0.03b	0.03b	0.03b	0.03b	3.5abc	3.3bc
LSD	**6.07**		**0.07**		**0.02**		**2.63**		**0.02**		**0.04**		**1.44**	

Key: WV= Wheat varieties, V_1= Freeman, V_2= Overland, V_3= Overland FHB10, V_4= Panhandle, V_5= Robidoux, V_6= Ruth (NE10589), V_7= Settlers CL, V_8= NW07505, V_9= Mattern W4-265, V_{10}= McGill, V_{11}= RedHawk, V_{12}= SY-Wolf, L= Length, FW= Fresh weight, DW= Dry weight, I= Inoculated with pathogen, NI= Non-Inoculated (Control)

Table 3. Effect of inoculation of *R. solani* strain G256 on yield components of 12 wheat cultivars after three weeks.

WV	Shoot system						Root system						Leaves per plant	
	L (cm)		FW(g)		DW(g)		L (cm)		FW(g)		DW(g)			
	I	NI	I	NI	I	NI	I	NI	I	NI	I	NI	I	NI
V_1	13.37e	14.30c-e	0.07de	0.05e	0.03ef	0.03f	11.62b	14.88a	0.02b	0.02b	0.02c	0.02bc	2.3f	3.0ef
V_2	16.88a-e	16.88a-e	0.09b-e	0.10b-e	0.03d-f	0.05a-e	13.70ab	13.78a	0.02b	0.03b	0.05ab	0.09a	3.0ef	3.5c-e
V_3	20.62a	18.25a-e	0.13a-c	0.13a-c	0.06ab	0.06ab	13.63ab	14.55a	0.02b	0.02b	0.02bc	0.02bc	4.5a-c	4.5a-c
V_4	20.85a	19.13a-d	0.17a	0.11b-e	0.06a	0.05a-e	13.55ab	14.37a	0.02b	0.05a	0.02bc	0.02bc	5.0a	4.8ab
V_5	19.60a-c	19.38a-d	0.11a-d	0.11a-d	0.05a-e	0.05a-c	14.13a	14.13a	0.02b	0.02b	0.02bc	0.02bc	4.3a-d	4.5a-c
V_6	16.23a-e	18.28a-e	0.09b-e	0.11a-d	0.04a-f	0.05a-d	14.50a	14.15a	0.02b	0.03b	0.02bc	0.03bc	4.0a-e	3.8b-e
V_7	16.68a-e	19.75ab	0.09b-e	0.11a-d	0.04b-f	0.05a-e	14.00a	13.95a	0.02b	0.03b	0.02bc	0.02bc	4.0a-e	4.0a-e
V_8	16.87a-e	15.55a-e	0.10b-e	0.08c-e	0.05a-e	0.03f	14.45a	14.62a	0.03b	0.02b	0.02bc	0.03bc	3.8b-e	3.5c-e
V_9	16.37a-e	17.63a-e	0.08de	0.10b-e	0.04c-f	0.05a-e	13.95a	14.20a	0.02b	0.03b	0.02bc	0.03bc	3.0ef	3.3d-f
V_{10}	14.85b-e	14.13de	0.08c-e	0.06de	0.03d-f	0.04c-f	14.00a	14.43a	0.02b	0.02b	0.02bc	0.02bc	3.0ef	3.3d-f
V_{11}	19.20a-d	19.50a-d	0.10b-e	0.14ab	0.05a-f	0.05a-e	13.88a	14.08a	0.02b	0.03b	0.02bc	0.02bc	3.3def	3.3d-f
V_{12}	17.30a-e	16.13a-e	0.10b-e	0.10b-e	0.05a-e	0.04a-f	14.13a	13.38ab	0.02b	0.03b	0.02bc	0.03bc	3.5c-e	3.3d-f
LSD	**5.39**		**0.05**		**0.02**		**2.08**		**0.02**		**0.04**		**1.17**	

Key: WV= Wheat varieties, V_1= Freeman, V_2= Overland, V_3= Overland FHB10, V_4= Panhandle, V_5= Robidoux, V_6= Ruth (NE10589), V_7= Settlers CL, V_8= NW07505, V_9= Mattern W4-265, V_{10}= McGill, V_{11}= RedHawk, V_{12}= SY-Wolf, L= Length, FW= Fresh weight, DW= Dry weight, I= Inoculated with pathogen, NI= Non-Inoculated (Control)

Table 4. Effect of inoculation of *R. solani* strain W1624 on yield components of 12 wheat cultivars after three weeks.

WV	Shoot system						Root system						Leaves per plant	
	L (cm)		FW(g)		DW(g)		L (cm)		FW(g)		DW(g)			
	I	NI	I	NI	I	NI	I	NI	I	NI	I	NI	I	NI
V_1	12.75f	23.38a-c	0.09i	0.15e-i	0.03f	0.06b-f	13.83a	14.75a	0.02c-f	0.03a-d	0.01g	0.03a-c	2.3f	3.8b-f
V_2	21.63a-e	25.30a	0.25b-e	0.32ab	0.06b-f	0.08a-c	15.10a	15.87a	0.02c-f	0.02c-f	0.02c-g	0.02b-f	4.8a-d	5.8a
V_3	21.83a-e	24.88ab	0.22b-h	0.37a	0.06b-e	0.09a	15.63a	15.30a	0.03a-c	0.03a-d	0.03a-c	0.03a-c	5.0a-c	5.8a
V_4	17.08c-f	20.28a-e	0.16d-i	0.16d-i	0.04d-f	0.05c-f	10.78b	15.62a	0.02c-f	0.04ab	0.02c-g	0.03a-c	3.8b-f	3.8b-f
V_5	15.63ef	20.45a-e	0.11g-i	0.18c-i	0.04ef	0.06c-f	13.78a	16.40a	0.02c-f	0.03a-d	0.02c-g	0.03a-c	2.8ef	4.5a-d
V_6	21.25a-e	18.55a-f	0.22b-g	0.17d-i	0.07b-d	0.06b-f	14.90a	15.63a	0.03a-c	0.05a	0.02c-g	0.04a	4.8a-d	3.8b-f
V_7	22.78a-d	23.70a-c	0.19c-i	0.21c-h	0.06b-f	0.06b-f	16.48a	15.30a	0.02c-f	0.03a-d	0.02c-g	0.03a-c	4.8a-d	4.5a-d
V_8	18.25b-f	17.38c-f	0.18c-i	0.21b-h	0.06c-f	0.07b-d	15.13a	15.50a	0.03a-c	0.03a-d	0.02c-g	0.03a-c	4.5a-d	5.3ab
V_9	18.70a-f	20.88a-e	0.13f-i	0.18c-i	0.05d-f	0.06b-f	15.13a	15.40a	0.03a-c	0.03a-d	0.03a-c	0.03a-c	3.8b-f	3.3d-f
V_{10}	16.58d-f	19.72a-e	0.115hi	0.16d-i	0.04d-f	0.05c-f	14.90a	15.75a	0.02c-f	0.03a-d	0.02c-g	0.03a-c	3.5c-d	4.3a-e
V_{11}	23.75a-c	23.40a-c	0.26a-d	0.28a-c	0.08a-c	0.08ab	14.15a	14.58a	0.02c-f	0.03a-d	0.02c-g	0.03a-c	5.3ab	5.5a
V_{12}	22.60a-d	21.75a-e	0.09i	0.24b-f	0.07b-d	0.07b-d	15.18a	15.38a	0.03a-c	0.03a-d	0.03a-c	0.03a-c	4.5a-d	4.8a-d
LSD	**6.69**		**0.11**		**0.03**		**2.77**		**0.01**		**0.01**		**1.51**	

Response of wheat lines to growth parameters against W433: In this research project, 12 wheat cultivars were tested against root rot causing pathogen *F. graminearum* strain W433. The pathogen was inoculated at the time of sowing. After three weeks, yield components that included length of root and shoot, fresh and dry weight of root and shoot were measured. All artificially inoculated WC showed almost similar shoot length (L) according to statistical evaluation. Fresh weight (FW) of shoot was slightly variable between treated and non-treated varieties but dry weight (DW) indicated similar significant distribution among inoculated and non-inoculated trails. Similarly, root length, root FW and DW of wheat lines appeared significantly similar (Table 2). Impacts of W433 on number of leaves/plant was also documented. There was no significant difference appeared between treated and non-treated trails. It means that the pathogen did not decrease biomass of leaf, root and shoot of wheat plants (Table 2).

Response of wheat lines to growth parameters against G256: Responses of yield components of 12 WC were also measured after three and six week's inoculation period of *R. solani* strain G256. Shoot length of five artificially inoculated WC as V_1 (Freeman), V_6 (Ruth), V_7 (Settlers), and V_9 (Mattern) showed less weight when compared with control. Other seven WC indicated more or less similar shoot length. Shoot FW indicated that V_6 (Ruth), V_7 (Settlers), V_9 (Mattern) and V_{11} (RedHawk) reduced weight after pathogen inoculation. On the other hand, reduced shoot DW of five WC (V_2, V_6, V_7, V_9, V_{10}) were also measured in artificially inoculated (G256) trails. Inoculated and non-inoculated trails showed significant difference in root length of V_1 (Freeman) and slightly difference in V_3 (Overland FHB10) and V_4 (Panhandle). Other nine WC were showed significantly similar root length as indicated in Table 3.

Root FW of all varieties indicated similar mass expect V_4. The variety V_4 reduced weight significantly after attack of *R. solani*. On the other hand, DW of root was also observed similar in all WC except V_2. Counting of leaves per plant among WC also exhibited same distribution between treated and non-treated trials. Over all, it was observed that the pathogen G256 decreased the biomass of leaf, root and shoot of some wheat lines (Table 3).

Response of wheat lines to growth parameters against W1624: Response of wheat lines against W1624 were analyzed (Table 4). Length of nine WC was reduced after W1624 inoculation. Similarly, ten inoculated WC were reduced FW of shoot as compared with control. Dry weight of shoot was also reduced of seven varieties after inoculation of W1624 strain. On the other hand, root system also showed variation between treated and non-treated samples. Out of 12 WC, 5 showed reduced root length. Root FW of seven wheat varieties was reduced as compared to control. Dry weight of root was also affected by the attack of *R. solani* strain W1624. Reduced root was observed in eight WC (DW). Leaves per plant (LPP) were also calculated. It was observed that there were five WC having reduced LPP between NI and I wheat trails under greenhouse conditions as mentioned (Table 4). As we compared Rhizoctonia strain G256 and W1624, it was

observed that strain W1624 was more virulent to cause root rot disease and reduce more biomass of yield components (Table 4).

Statistical analysis of three fungal pathogenic strains (W433, G256 and W1624) of root dry weight (RDW) of inoculated and non-inoculated experimental trails of 12 wheat cultivars were mentioned in Fig. 3A, B, C. Wheat cultivar 'Overland' indicated the highest significant difference with reface to DW in Fusarium strain W433. Similarly, Rhizoctonia strain G256 showed the highest significant difference in WC 'Overland'. On the other hand, Rhizoctonia strain W1624 showed less significant difference among wheat cultivars. All WC reduced dry weight of root more or less equally and shown almost statistically equal distribution (Fig. 3A, B, C).

Discussion

The current study focused on the management of root rot diseases of 12 wheat cultivars through development of resistance caused by one *F. graminearum* strain W433 and two *R. solani* strains G256 and W1624. The development of resistant WCs is one of the cheapest, safe and durable methods for the control of fungal diseases. Many scientists worked on the development of resistance of WCs against fungal diseases through screening (Anaso *et al*., 1984; Corrazza *et al*., 1987; Mishra *et al*., 1992; Harlapur *et al*., 1993; Ahmed *et al*., 2009).

Response of wheat cultivars against root rot pathogens: Response of 12 wheat cultivars (WC) against 3 fungal strains were determined based on 1-8 rating scale. It was observed that WC Freeman and NW07505 were moderately resistant while Overland, Overland FHB10, Panhandle, Ruth, Mattern, RedHawk and SY-Wolf were susceptible against *F. graminearum* strain W433. These results were supported by Mukankusi, (2008). He discussed that variation appeared in genes actions and numbers. These variations governing resistance in genotypes. Accumulation of these resistant genes in different cultivars were suggested as a way to increase levels of resistance against Fusarium root rot.

Rhizoctonia strain G256 was produced moderate resistance to Overland FHB10, Panhandle and NW07505. It was susceptible to Freeman, Ruth, Mattern and McGill. Similarly, *Rhizoctonia* strain W1624 showed minimum disease severity against Freeman, NW07505 and SY-Wolf while more severity appeared against Overland, Robidoux, Ruth and Mattern. Statistically, it was observed that some cultivars showed difference and some indicated significantly same disease severity (DS). It was also observed that DS appeared similar after three and six-week's interval. Therefore, in this manuscript, we mentioned only the DS that was calculated after harvesting of three-week period. These findings were showed similar expression to the previous findings of Okubara *et al*., (2009) and Paulitz & Schroeder (2005). Tolerance against many isolates of each pathogen species indicates a vast activity. The tolerance limits of 100 ppg that were observed in our greenhouse studies exceeded the 20– 85 ppg that were associated with Rhizoctonia root diseases of cereals in the field (Paulitz and Schroeder 2005). The most reliable combination of variables for tolerance was deemed the disease severity rating. Disease severity ratings were generally observed by infected roots (Okubara *et al*., 2009). Oros *et al*., (2013) evaluated the responses of 19 wheat varieties against soil borne Rhizoctonia infection that were cultivated in Hungary. The inhibition of development of survivors in Rhizoctonia infested soil correlated with overall susceptibility of variety concerned. Some varieties are proved less susceptible but none of the varieties could be certified as tolerant. These justifications were supported the current study.

Response of wheat lines to growth parameters against fungal strain W433: Responses of 12 WC were tested against root rot causing pathogen *Fusarium graminearum* strain W433 and measured their yield components. Firstly, yield components measured after three weeks intervals that include length of root and shoot, fresh and dry weight of root and shoot. It was observed that all artificially inoculated WC showed almost similar shoot length (L) according to statistical analysis. Fresh weight (FW) of shoot was slightly variable between treated and non-treated WC but dry weight (DW) showed similar significant distribution between inoculated and control. Similarly, root length, root FW and DW of WC indicated significantly similar distribution. Counting of leaves per plant (LPP) did not show significant difference between treated and non-treated samples. It means that the pathogen did not decrease leaf numbers, root weight and shoot weight of WC significantly.

Secondly, all yield components were measured again after harvesting of six-week interval. It was observed that shoot length exhibited difference between treated and non-treated experimental trails. Inoculated plants indicated less shoot (L) as compared to control. As we measured FW of shoot, three varieties showed slight difference among treated and non-treated samples. Other nine WC showed similar FW. According to shoot DW, out of twelve WC, six indicated slight reduced weight after W433 strain inoculation. The root length of two WC slightly reduced as compared to control. Other, ten WCs showed significantly similar root length. Root FW of all wheat germplasm exhibited significantly same distribution except Panhandle (V_4). Similarly, DW of Overland (V_2) root indicated significantly reduced weight in comparison to control. Overall, it was estimated that all artificially inoculated WCs decreased biomass as compared to control (untreated). It means that W433 strain of *F. graminearum* was virulent for root rot disease against different wheat lines and it was gradually increased virulence against wheat cultivars. These results were supported by Navarro *et al*., (2003). They described that the most effective control measure of Fusarium root rot in common bean was through the deployment of resistant cultivars.

Response of wheat lines to growth parameters against fungal strain G256: Responses of yield components of 12 WC were also measured after three and six week's inoculation period of *R. solani* strain G256. Shoot length of four WCs as Freeman, Ruth, Settlers and Mattern showed less weight when

compared with control. Shoot FW indicated reduced weight in WCs Ruth, Settlers, Mattern and RedHawk after pathogen inoculation. Reducing shoot DW of five WCs were also measured in artificially inoculated G256 trails. Inoculated and non-inoculated trails showed significant difference in root length of WCs Freeman and less significant difference was observed in Overland FHB10 and Panhandle. Other nine WC depicted significantly similar root length. Root FW of all varieties had similar weight expect V_4. The variety V_4 reduced weight significantly after attack of *R. solani* (G256). On the other hand, DW of root was also observed similar in all WC except V_2. Counting of leaves per plant among WC were also showed same distribution between treated and non-treated trails. Over all, it was observed that the pathogen strain G256 decreased biomass of leaf, root and shoot of some WC. Okubara *et al*., (2009) supported these results. They described that gross measurements of root or seedling fresh weight and shoot length were not consistent parameters of susceptibility and resistance. When pathogen damaged roots became stunted and thickened with only minor loss of root mass but lateral roots growth was severely reduced (Okubara *et al*., 2009).

Yield components were also calculated after six-week inoculation period of fungal strain G256. Shoot length of WCs (NW07505) decreased between treated and non-treated trails. According to statistical analysis, other 11 WC showed same distribution of root length. According to shoot FW, there was no distinct difference between NI trials and I. Similarly, DW of shoot showed similar distribution except Freeman. Root length of treated and non-treated wheat plants exhibited similar distribution except V_9 and V_{10}. These two varieties had reduced root length after G256 inoculation. Fresh weight of root was also decreased in the only variety V_9. Three WC showed reduced DW after six-week inoculation period of fungal strain G256. These findings were correlated with previous studies (Kulkarni & Chopra, 1982). The specificity of *R. solani* isolates were evaluated on flax cultivars because the concept of specificity in host-pathogen interaction helped to understand plant diseases and their management.

Responses of wheat lines to growth parameters against fungal strain W1624: Responses of 12 wheat lines against W1624 were also observed after three weeks interval. Lengths of nine WC were reduced after W1624 inoculation. Similarly, ten inoculated WC were reduced FW of shoot as comparison to control. Dry weight of shoot was also reduced by seven varieties. Similarly, root system also exhibited variation between treated and non-treated (control) samples. Root FW of seven WC were reduced as compared to control. Dry weight of root was also affected by the attack of *R. solani* strain W1626. It was also observed that five WC reduced LPP between NI and I wheat trails under greenhouse conditions. These results were related to the study of Aly *et al*., (2013). Aly research group tested 24 isolates of *R. solani* and evaluated 10 flax cultivars under greenhouse conditions through pathogenicity test. Survival, plant height and dry weight were used as criteria to evaluate pathogenicity. Analysis of variance (ANOVA) showed that the cultivar was a highly significant source of variation in all the tested parameters.

Many scientists tested wheat cultivars and catogerized WCs as resistant or susceptibe in the world. Anaso *et al.,* (1984) tested 14 cultivars against foot and root rot in pot soil. All 14 cultivars showed mild to moderate susceptibility to *Drechslera spp.* and *Fusarium spp.* Karaw & Singh (1975) tested 15 wheat varities against *P. graminicolum, H. sativum, S. rolfsii* and *Fusarium spp.* All of them were graded as susceptible. Kulkarni *et al*., (1978) tested 35 wheat varieties against *S. rolfsii* and identified 4 varieties as resistant. Ahmed & Bakr (1991) identified only 9 wheat germplasm as resistant to *S. rolfsii*, out of 140 germplasm tested under inoculated conditions.

Comparative assessment of Rhizoctonia strain G256 and strain W1624 indicated significant difference. It was observed that strain W1624 was more virulent in comparison to G256. It caused more root rot DS and reduced more biomass of yield components. These findings were supported by Mohammadi & Kazemi (2002) and Michereff *et al*., (2008). Oros *et al*., (2013) also tested wheat varieties against Rhizoctonia strain. He proved some WCs were less tolerant while few exhibited low susceptibility against Rhizoctonia strain. He also evidenced that the response of *T. monococcum* and *T. turgidum* was similar to more tolerant *T. aestivum* cultivars. Unfortunately, none of the test plants tolerated the majority of Rhizoctonia strains at high degree.

Conclusion

It was concluded that all three fungal strains were virulent to cause root rot diseases in wheat cultivars. It was determined through pathogenicity test against 12 WC under controlled conditions of greenhouse. It was estimated that WC Freeman and NW07505 were moderately resistant while Overland, Overland FHB10, Panhandle, Ruth, Mattern, RedHawk and SY-Wolf were susceptible against *F. graminearum* strain W433. While Rhizoctonia strain G256 produced moderate resistance to Overland FHB10, Panhandle and NW07505. It was susceptible to Freeman, Ruth, Mattern and McGill. Similarly, *Rhizoctonia* strain W1624 exhibited minimum disease severity against Freeman, NW07505 and SY-Wolf while more severity appeared against Overland, Robidoux, Ruth and Mattern. Statistically, it was observed that some cultivars shown differences and some indicated significantly same disease severity (DS). It was observed that all artificially inoculated WC decreased biomass, in comparison to control due to virulence in favor of root rot diseases and it was gradually increased virulence against wheat cultivars with increased time duration. Rhizoctonia strain G256 also decreased biomass of leaf, root and shoot of some WC. When we compared both Rhizoctonia strains between them it was observed that strain W1624 was more virulent as compared to G256. It caused more root rot DS and reduced more biomass of yield components.

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