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EFFECT OF BACTERIAL WILT ON SOME PHYSIOLOGY OF GROUNDNUT VARIETIES (*Arachis hypogaea*. L)

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Abstract

Bacterial wilt caused by *Ralstonia solanacearum* is an important groundnut pathogen that caused rapid wilting and death of entire groundnut without any yellowing or spotting of leaves. The aim was to determined the wilt disease effect on chlorophyll and photosynthesis on selected groundnut varieties (*Arachis hypogaea*.L). Eleven varieties of groundnut were used; the experiment was arranged in Randomized Complete Block Design with four treatments; *R. solanacearum* inoculation through leaves, seed, stem and control with three replication. Result for chlorophyll content showed that there is a significance difference between treatment and variety, but there is no significance difference between treatments by variety interaction at 7days after inoculation. Highly significance difference between treatments, variety and treatments by variety interaction was observed at 28 and 49days after inoculation. The result for photosynthesis showed a significance difference between treatment and variety, but there is no significance between treatment and variety at 14days after inoculation. Highly significance difference was observed between treatments, variety and treatments by variety interaction at 21days. SAMNUT 23, Bahaushiya and Kwankwaso recorded high disease incidence after foliar, seed and stem inoculation respectively.

Keywords: Physiology, Bacterial wilt, chlorophyll, photosynthesis, Groundnut

Introduction

Groundnut is an important crop for oil and protein production in tropical and subtropical regions of the world, Africa is region of groundnut production with growing areas of 9.04million ha (FAO, 2007). The bacterial wilt caused by *R. solanacearum* is a destructive soil borne groundnut disease; it causes reduction in production of groundnut to at least 10%. *R. solanacearum* has a wide host range expanding over more than 200 plant species (Hayward, 1991). The process of *R. solanacearum* infecting plant had been well characterized in model crop briefly, it penetrate into cortical tissue of host roots, colonized and explode in numbers and cause a sudden deadly wilt of plant (Van Overbeek *et.al.,* 2002 and Fujie *et.al.,* 2010). Infection in young plants results in rapid wilting of stems and foliage, while leaves retain their green colour (Mehan *et.al.,* 1994). Wilt symptoms can be observed 3weeks after planting. The peak of occurrence appears in 40-50days after planting (Wang and Gai, 1999). In less affected field, 10-30% groundnut plants show symptoms, causing more than 20% yield reduction. This study was to determine the effect of wilt disease caused by *R. solanacearum* on chlorophyll content and photosynthesis rate of selected groundnut varieties.

Experimental site

The study was conducted under rain fed condition at the Department of Agronomy research farm, Bayero University Kano, Nigeria which lies latitude 11°58N and longitude 8°30E.

Seed collection

Eleven varieties of groundnut were used for this study. 7 improved varieties (SAMNUT 10, SAMNUT 11, SAMNUT 21, SAMNUT 22, SAMNUT 23, SANMUT 24 and SAMNUT 25) and 4 local varieties (Bahaushiya, EX-Dakar, Maibargo and Kwankwaso).

Experimental design

The experiment was arranged in Randomized Complete Block Design with three replications and four treatments; leaf inoculation, seed inoculation, stem inoculation and control.

Methodology

The experiment involved infecting the leaves, stems and seeds of *A. hypogaea* with *R. solanacearum* to determine the effects on chlorophyll(Feruse and Arkersivora, 2001) content after 7, 28 and 49days and photosynthesis rate (Rowland *et.al.,* 2005) after 7, 14 and 21days of infection. Records of disease manifestation was measured in terms of wilting of plants as follows: (1) No symptoms, (2) One leaf wilted at inoculation point, (3) Two to three leaves wilted, (4) Four or leaves wilted, (5) Whole plant wilted (Mehan and McDonald, 1995).

Isolation of *Ralstonia solanacearum*

Ralstonia solanacearum was isolated from soil sample collected from tomato fields. *R. solanacearum* was cultured on Tetrazolium chloride and purified on Nutrient agar at 30°C for 24hrs (French *et.al.,* 1995).

Inouculum prepation

Bacterial growth on nutrient agar was washed on a slant in 5ml distilled water and mixed well whereby a bacterial population of $1X10^{8}cfum^{-1}$ was obtained. 100µl (0.1ml) of *R. solanacearum* was used for stem inoculation. Also $6X10^{8}cfum^{-1}$ of *R. solanacearum* was used for seed and leaves inoculation (Mehan and McDonald, 1995).

Statistical analysis

Collected data was subject to Analysis of Variance (ANOVA) and mean separation was done using Least Significance Difference (LSD) at 0.05 level using GenStat version 16.0.

Result

Chlorophyll content

The results for chlorophyll content after *Ralstonia solanacearum* inoculation are presented in Table 1. The statistical analysis showed that there is significant differences (P<0.05) between treatment and variety, but there is no significant difference (P>0.05) between treatment by variety interaction at 7days. The result showed that at 7days after foliar inoculation, variety SAMNUT 22 (27.03) and SAMNUT 11 (27.17) recorded high chlorophyll content, while Ex-Dakar (15.03), SAMNUT 24 (16.36) and SAMNUT 25 (17.33) varieties recorded low chlorophyll content. After seed inoculation at 7days, SAMNUT 24 (23.90) and SAMNUT 21 (23.67) recorded high chlorophyll; SAMNUT 25 (13.90) and Kwankwaso (14.47) recorded low chlorophyll content. SAMNUT 23 (28.57) and Bahaushiya (26.83) recorded high chlorophyll content, while SAMNUT 10 (14.53) recorded low content of chlorophyll after stem injection at 7days.

At 28days after disease inoculation, the result of statistical analysis indicates a highly significant differences (P<0.05) for treatment, variety and treatment by variety interaction. At 28days after foliar inoculation, SAMNUT 25 (91.62) and Maibargo (91.38) varieties recorded high chlorophyll content, SAMNUT 21 (61.61) and SAMNUT 22 (61.95) recorded low chlorophyll content. SAMNUT 25 (75.39) and SAMNUT 22 (75.32) recorded high chlorophyll content while Kwankwaso (70.68) recorded low chlorophyll content after seed inoculation at 28days. Kwankwaso (72.73) and Bahaushiya (72.62) recorded high chlorophyll while SAMNUT 10 (62.53) recorded low chlorophyll content at 28days after stem injection.

Statistical analysis for chlorophyll content at 49days shows a highly significant differences (P<0.05) for treatment, variety and treatment by variety interaction. SAMNUT 24 (76.49) and SAMNUT 25 (76.10) recorded high chlorophyll content while Maibargo (71.56) and Ex-Dakar (71.26) recorded low chlorophyll content after foliar inoculation at 49days. After seed inoculation at 49days, SAMNUT 10 (72.16) and SAMNUT 11 (72.46) recorded high chlorophyll content, while Kwankwaso (54.60) recorded low content of chlorophyll. At 49days after stem injection, SAMNUT 23 (74.58) and SAMNUT 24 (73.22) recorded high chlorophyll content; Ex-Dakar (70.52) and Kwankwaso (70.84) recorded low chlorophyll content.

Photosynthesis rate

The results for photosynthetic active radiation (PAR) after *Ralstonia solanacearum* inoculation are presented in

Table 2. The statistical analysis result showed that there is no significant differences (P>0.05) between treatment, variety and treatment by variety interaction at 7days after inoculation. The result showed that the photosynthetic active radiation of some groundnuts varieties after *Ralstonia solanacearum* inoculation at 7days recorded same value (10) for foliar inoculation, seed inoculation and stem injection.

At 14days, the result showed that there is significant difference (P<0.05) between treatment and variety, but there is no significant differences (P>0.05) between treatment by variety interaction. SAMNUT 25 (10.67) and SAMNUT 24 (10.33) recorded highest photosynthetic active radiation

Table 1: Treatment by Variety interaction for chlorophyll content (µmolm^{-2}) at 7, 28 and 49days after *Ralstonia solanacearum* inoculation

	7days				28 days				49 days			
Variety	C	FS	SD	ST	C	FS	SD	ST	C	FS	SD	ST
Bahaushiya	24.20 ±1.00	22.86 ±2.04	19.90 ±1.62	25.8± 2.06	81.10 ±2.44	90.82 ±0.26	73.93 ±0.34	71.38 ±0.61	73.31 ±0.60	72.01 ±0.08	57.88 ±0.36	72.32 ±0.12
Ex-Dakar	17.16 ±1.06	15.03 ±0.52	18.46 ±0.79	19.03 ±1.16	88.90 ±0.17	90.84 ±0.11	74.44 ±0.26	71.13 ±0.79	72.33 ±0.43	71.26 ±0.76	56.34 ±0.77	71.22 ±0.29
Kwankwaso	17.03 ±1.29	19.95 ±0.59	14.46 ±0.41	22.15 ±1.61	90.86 ±0.37	90.19 ±0.10	70.68 ±0.20	72.57 ±0.38	71.98 ±0.44	72.69 ±0.06	54.59 ±0.30	70.35 ±0.39
Maibargo	24.46 ±2.13	21.03 ±0.65	22.00 ±1.52	21.23 ±0.81	76.81 ±3.24	91.37 ±0.25	74.35 ±0.27	71.80 ±0.23	72.98 ±0.10	71.56 ±0.13	58.19 ±0.84	72.53 ±0.09
SAMNUT10	11.66 ±1.43	17.15 ±1.77	21.90 ±0.50	13.95 ±0.34	68.70 ±0.52	69.32 ±3.69	72.68 ±0.40	62.71 ±0.09	74.33 ±0.24	73.79 ±0.32	72.16 ±0.09	72.91 ±0.42
SAMNUT11	10.60 ±0.60	27.16 ±0.44	20.10 ±1.24	23.36 ±2.06	68.80 ±0.62	62.76 ±0.14	75.14 ±0.26	63.34 ±0.35	75.85 ±0.18	74.38 ±0.28	72.46 ±0.07	72.43 ±0.24
SAMNUT21	18.66 ±3.02	19.80 ±0.99	23.66 ±1.20	24.43 ±1.41	68.31 ±0.48	61.61 ±0.44	74.13 ±0.39	62.69 ±0.50	74.08 ±0.20	73.94 ±0.08	61.04 ±2.21	73.29 ±0.11
SAMNUT22	15.06 ±1.39	27.03 ±4.99	17.16 ±0.84	21.66 ±0.75	68.49 ±0.53	61.95 ±0.15	75.31 ±0.47	66.94 ±1.12	73.12 ±0.24	75.56 ±0.52	61.14 ±1.18	70.91 ±0.38
SAMNUT23	18.06 ±1.64	26.96 ±1.71	17.90 ±0.83	24.23 ±1.90	70.35 ±0.10	71.17 ±4.67	74.37 ±0.14	70.34 ±0.26	73.10 ±0.78	75.34 ±0.45	57.79 ±0.50	75.21 ±0.67
SAMNUT24	14.90 ±0.68	16.36 ±0.59	23.90 ±2.94	27.86 ±2.32	69.85 ±0.12	89.83 ±0.15	74.08 ±0.23	71.12 ±0.08	75.44 ±0.35	76.49 ±0.55	59.19 ±0.21	73.65 ±0.63
SAMNUT25	10.16 ±1.02	17.33 ±0.93	13.90 ±1.34	19.73 ±1.12	69.89 ±0.10	91.61 ±0.20	75.38 ±0.68	71.54 ±0.47	73.62 ±0.78	76.10 ±0.25	58.59 ±0.47	71.21 ±0.97
Mean	16.54	20.96	19.39	22.13	74.73	79.22	74.04	68.68	73.64	73.92	60.85	72.36
S.E±	1.45	1.35	1.02	1.13	2.56	4.09	0.40	1.19	0.36	0.55	1.79	0.42
Treatment		0.0003				0.0001				0.0001		
Variety		0.0011				0.0001				0.0001		
Treat* variety		NS				0.0001				0.0001		

Key; C=Control, FS=Foliar inoculation, SD=Seed inoculation, ST=Stem injection, ±=Standard error, LSD=Least significance difference

Table 2: Treatment by Variety interaction for photosynthesis at 7, 14 and 21days after *Ralstonia solanacearum* inoculation

		7days				14days				21days		
Variety	C	FS	SD	ST	C	FS	SD	ST	C	FS	SD	ST
Bahaus	10.00	10.00	10.00	10.00	7.66±	8.00±	8.66±	8.66±	9.00±	9.00±	8.66±	9.00±
hiya	±0.00	±0.00	±0.00	±0.00	0.33	0.57	0.16	0.16	0.57	0.76	0.60	0.00
Ex-	10.00	10.00	10.00	10.00	8.00±	7.66±	9.00±	7.66±	8.66±	8.60	7.66±	8.00±
Dakar	±0.00	±0.00	±0.00	±0.00	0.28	0.16	0.50	0.57	0.33	±0.60	0.16	0.28
Kwank	10.00	10.00	10.00	10.00	7.33±	8.00±	7.33±	7.50±	10.00	9.50±	9.33±	7.50±
waso	±0.00	±0.00	±0.00	±0.00	0.16	0.40	0.16	0.37	±0.50	0.20	0.43	0.16
Maibar	10.00	10.00	10.00	10.00	8.00±	9.00±	7.66±	9.33±	10.33	7.33±	10.66	9.00±
go	±0.00	±0.00	±0.00	±0.00	0.00	0.00	0.16	0.72	±0.43	0.16	±0.43	0.50
SAMN	12.00	10.00	10.00	10.00	8.66±	8.50±	8.33±	8.00±	11.66	9.75±	10.33	10.00
UT10	±0.00	±0.00	±0.00	±0.00	0.16	0.50	0.33	0.40	±0.16	0.59	±0.43	±0.40
SAMN	10.00	10.00	10.00	10.00	7.66±	8.33±	8.66±	8.66±	11.00	9.66±	9.33±	10.33
UT11	±0.00	±0.00	±0.00	±0.00	0.16	0.33	0.16	0.16	±0.28	0.43	0.66	±0.60
SAMN	10.00	10.00	10.00	10.00	6.00±	9.00±	9.33±	7.66±	11.33	10.66	7.33±	9.33±
UT21	±0.00	±0.00	±0.00	±0.00	0.00	0.50	0.43	0.16	±0.33	±0.43	0.16	0.60
SAMN	10.00	10.00	10.00	10.00	8.00±	10.00	9.33±	7.66±	11.66	9.66±	10.00	9.00±
UT22	±0.00	±0.00	±0.00	±0.00	0.00	±0.57	0.72	0.33	±0.16	0.43	±0.00	0.50
SAMN	10.00	10.00	10.00	10.00	8.00±	10.00	9.33±	9.00±	11.33	9.33±	10.66	9.66±
UT23	±0.00	±0.00	±0.00	±0.00	0.16	±0.28	0.33	0.28	±0.16	0.33	±0.16	0.16
SAMN	10.00	10.00	10.00	10.00	11.00	10.33	10.00	10.33	10.33	9.00±	11.33	10.66
UT24	±0.00	±0.00	±0.00	±0.00	±0.28	±0.16	±0.50	±0.43	±0.60	0.28	±0.16	±0.33
SAMN	10.00	10.00	10.00	10.00	9.66±	10.66	9.50±	9.66±	9.66±	9.00±	9.33±	11.00
UT25	±0.00	±0.00	±0.00	±0.00	0.16	±0.43	0.60	0.33	0.16	0.57	0.43	±0.00

Means	10.18	10.00	10.00	10.00	8.17	9.04	8.83	8.55	10.45	9.22	9.51	9.40
S.E±	0.18	0.00	0.00	0.00	0.38	0.31	0.24	0.28	0.31	0.25	0.37	0.32
Treatment		NS				0.044				0.0006		
Variety		NS				0.0001				0.0023		
Treat* variety		NS				NS				0.0021		

Key: C=Control, FS=Foliar inoculation, SD=Seed inoculation, ST=Stem injection, ±=Standard error, LSD=Least Significance difference

after foliar inoculation of *Ralstonia solanacearum* at 14days while Ex-Dakar (7.67) and Kwankwaso (10.67) recorded low photosynthetic active radiation at 14days. Seed inoculated SAMNUT 24 (10.00) recorded highest photosynthetic active radiation, kwankwaso (7.33) and Maibargo (7.66) varieties recorded low photosynthetic active radiation after inoculation at 14days. SAMNUT 24 (10.67) recorded highest photosynthetic active radiation after stem injection, ex-Dakar (7.33) and SAMNUT 21 (7.33) recorded low photosynthetic active radiation after stem injection inoculation at 14days.

The result also showed that there is significant difference (P<0.05) between treatment, variety and treatment by variety interaction at 21days after inoculation. At 21 days, SAMNUT 21 (10.66) and Maibargo (7.33) recorded highest and lowest photosynthetic active radiation respectively after inoculation of *Ralstonia solanacearum* by foliar spray inoculation. SAMNUT 24 (11.33) recorded highest photosynthesis rate, while Ex-Dakar (7.33) and SAMNUT 21 (7.33) recorded low photosynthetic active radiationat 21days after seed inoculation of *Ralstonia solanacearum*. Stem injected SAMNUT 11 (11.33) recorded highest photosynthetic active radiation at 21days, while Kwankwaso (7.33) recorded low photosynthetic active radiation after *Ralstonia solanacearum* inoculation.

Disease incidence

The result for disease incidence of some groundnut varieties infected with *Ralstonia solanacearum* bacteria are presented in Table 3. SAMNUT 23 (37.27%) recorded highest disease incidence at week 4 after disease inoculation through foliar inoculation, while SAMNUT 22 (10.90%) recorded low incidence. Varieties of groundnut infected with *Ralstonia solanacearum* by seed inoculation at week 4 shows that Bahaushiya (43.63%) variety recorded highest disease incidence while SAMNUT 24 (21.18%) recorded low incidence. At week four after disease inoculation by stem injection, Kwankwaso (45.45%) recorded highest disease incidence and Ex-Dakar (14.54%) recorded low disease incidence. SAMNUT 10 (1.81%) SAMNUT 11 (1.81%) recorded no symptoms.

Table 3: Disease incidence (%) of *Ralstonia solanacearum* on some groundnut varieties after 30days of inoculation

	Treatment		
Variety	FS	SD	ST
Bahaushiya	36.36	43.63	36.36
EX-Dakar	16.36	29.09	14.54
Kwankwaso	21.81	27.27	45.45
Maibargo	18.18	21.81	27.27
SAMNUT 10	1.81	1.81	1.81
SAMNUT 11	1.81	1.81	1.81
SAMNUT 21	27.27	21.81	43.63
SAMNUT 22	10.90	32.72	29.09
SAMNUT 23	37.27	32.72	21.81
SAMNUT 24	14.54	21.18	29.09
SAMNUT 25	32.72	21.81	29.09
Mean	19.91	23.24	25.45
S.E	3.78	3.78	4.41

Key: FS=foliar spray, SD=seed inoculation, ST=stem inoculation S.E=Standard error

Discussion

According to the results, high chlorophyll content was obtained at 28 days at all the inoculation techniques, with greatest reduction at early and late systemic infection coinciding with maximum symptom expression. This agrees with Naidu *et al.* (1984) who reported decrease in chlorophyll from early infection to establishment of systemic infection while working on peanut green mosaic virus, and Kutama *et al.* (2013) findings who reported reduction in chlorophyll could be due to destruction of spongy and intravascular tissues of plant body by pathogen and it could be due to susceptibility of the variety to disease.

Low chlorophyll content in this study was observed on seed and stem inoculated treatments with much decrease in later days of inoculation across all varieties, probably due to damage of photosynthetic apparatus by *Ralstonia solanacearum*. This finding corresponds with Rowland *et al.* (2005) who state that groundnut leaf chlorophyll was consistently lower in leaves with symptoms irrespective of plant disease category or cultivar, probably due to damage of leaf photosynthetic apparatus. Prashant *et al.*, (2009) reported that high chlorophyll content in leaves will in turn increase grain yield, while low chlorophyll content reduce grain yield.

Several groundnut varieties are infected with *Ralstonia solanacearum* pathogen but exhibited no symptoms, while some varieties recovered from initial infection and appeared normal; Lyerly *et al.* (2002) also reported a similar case on evaluation of *Arachis spp* for resistance to tomato spotted wilt virus. The result of present study however showed that the absence of visual symptoms does not mean the leaf physiology is not affected, since there is decrease in photosynthetic active radiationof symptomless varieties; same finding was also reported by Rowland *et al.* (2005).

Arias *et al.* (2003) found no changes in CO_2 fixation in infected sunflower until symptoms became evident; this is line with the present study where there are no changes in photosynthetic active radiation of groundnut varieties at early days of inoculation.

The present study reported that Kwankwaso has the highest disease incidence due to stem infestation; this is not the same with Muhammad and Bdliya (2011) result, which state that Ex-Dakar recorded high disease incidence. Stem injection has the highest disease on all varieties, followed by inoculated seed and foliar infestation. This however differ with Kutama *et al.*, (2013) result which found out that foliar infestation has high disease incidence, followed by stem injection and seed infestation. Varieties were considered resistant when no susceptible plants were found within the entry (SAMNUT 10 and SAMNUT 11), highly susceptible when no tolerant plants were plants present (Kwankwas, Maibargo and Bahaushiya) and moderately resistant when at least one plant within the entry has mild symptoms (SAMNUT 21, SAMNUT 22, SAMNUT 23, SAMNUT 24, SAMNUT 25 and Ex-Dakar). This agrees with the findings of Hayatu *et al.*, (2014) and Blackman and Eastrop (1999).

Conclusion

This study has shown that bacterial wilt caused by *Ralstonia solanacearum* had effect on chlorophyll and photosynthesis due to systemic infection which result in vascular occlusion of groundnut varieties after different treatments method. The present study also showed that tolerances have been found in one promising local varieties (EX-Dakar). Therefore, such variety should be used for development of resistant varieties against *R. solanacearum* that can perform in different environmental conditions.

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