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Response of robusta coffee populations to *Fusarium xylarioides* infection under screen house condition in Uganda

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Abstract

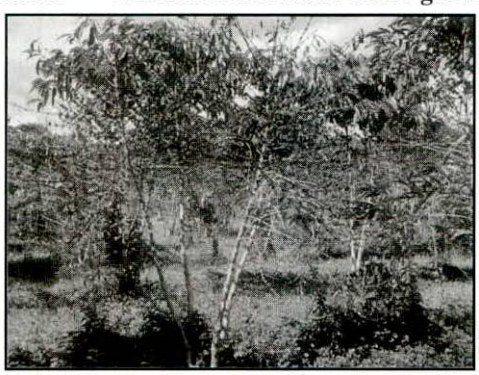
Seedlings of open pollinated seeds of 119 cultivars of robusta coffee (Coffea canephora) from coffee collections at Kituza and 8 progeny lines from wilt "hot spot" areas were inoculated to identify resistance to coffee wilt disease caused by F. xylariodes for effective control of the disease. The plants were inoculated with a field isolate of Fusarium xylarioides at a concentration of 1.3 x 10^6 spores per ml using root dip method for 12 hours. 15.4% of the seedlings survived first round of infection. The survivor seedlings were re-inoculated and 53% of them survived the second round of infection. The results showed variation in number of survivors between families and revealed presence of quantitative resistance to the coffee wilt disease among populations of robusta coffee.

Key words: *Robusta coffee, open pollinated seeds, seedlings,* Fusarium xylarioides*, coffee wilt disease, resistance.*

Introduction

Fusarium xylarioides Steyaert (imperfect state) or Gibberalla xylarioides Heim Saccs (perfect state) is the causal organism of coffee wilt disease. Coffee wilt disease is currently a major constraint to coffee production in Uganda. The disease appears to infect all species of coffee including wild species (Wrigley, 1988). It was first reported on Coffea exclesa in Central African Republic in 1927 (Muller, 1997). It devastated Coffea excelsa and Coffea abeokutae in Ivory Coast and Coffea canephora in Cameroon and the Democratic Republic of Congo between 1930 and 1950 (Muller, 1997; Flood, 1996). In 1958, Lejeune reported coffee wilt disease on arabica coffee in Ethiopia.

Plate 1: Wilt infected robusta coffee garden



During 1980s, new outbreaks of coffee wilt disease were reported in the Democratic Republic of Congo in areas bordering Uganda (Flood, 1996). The disease is also reported to still be an important constraint of arabica production in Ethiopia (Girma, 1997). In 1993, coffee wilt disease was reported in Uganda in Bundibugyo and Rukungiri districts bordering the Democratic Republic of Congo and at Kituza in Mukono district (Annon., 1996). It was reported in other parts of Mukono in 1994. A survey conducted in 1997 identified it in Kabarole, Mubende, Kibale, Hoima, Kiboga, Masindi Luwero, Mpigi, Kasese and Bushenyi districts. Currently the disease has spread and devastated coffee in all districts traditionally growing robusta coffee. It only affects robusta coffee.

Varietal resistance has been reported to be the most effective measure for controlling coffee wilt disease (Pieters et al, 1978). Muller (1997) reported the existence of resistance to coffee wilt disease among Coffea excelsa and C. canephora genotypes. Studies by Van der Graaff and Pieters (1978; 1980) indicate that

some arabica coffee lines in Ethiopia have horizontal resistance to the disease. Use of resistant varieties reduced tracheomycosis to a minor problem in Cameroon and Ivory Coast (Bakala, 1997; Boubacar, 1997). Studies conducted at the Coffee Research Institute (CORI) showed arabica coffee to be resistant to coffee wilt disease in Uganda (Musoli et al, 2001).

This paper presents results of similar studies conducted at CORI on robusta coffee to identify sources of resistance for developing resistant varieties.

Materials and methods

Seedlings of open pollinated seeds of 119 cultivars of robusta coffee in the germplasm collections at CORI and 8 individual tree survivors in wilt hotspots were raised in a coffee nursery at CORI following recommended nursery procedures. Seedlings of 5 clones recommended for commercial cultivation in Uganda were also raised for inclusion as controls. All the seedlings were tested for resistance against coffee wilt disease by inoculating with *Fusarium xylarioides.* The inoculations were carried out in a screenhouse when the plants were 6-9 months old.

The F. xylarioides inoculum was obtained from infected coffee stems. Pure cultures of *F. xylarioides* were prepared on potato dextrose agar (PDA) and sucrose nutrient agar (SNA) and a spore suspension was prepared and standardized at 1.3 x 10^6 spores per ml.

Only healthy looking seedlings were selected and used in the inoculation. The selected plants were removed from the pots and stripped off the potting soil and their roots cleaned under tap water. The cleaned plants were inoculated using the root dip method (Hakiza, 1999) for 12 hours in a standardized spore suspension of the field isolate of *F. xylarioides.* After the 12 hours, the plants were removed from the inoculum and repotted. The re-potted plants were arranged on a cemented floor in the screenhouse at Kituza in a randomized complete block design with three replicates and left to incubate at room temperature. All the plants were watered three times in a week, each plant receiving about 200 ml of water at each watering and they were monitored regularly for wilt symptoms. When the symptoms started showing, records were taken at bi-weekly intervals to assess the disease progress. Data recording was terminated when there were no new cases of seedlings with wilt symptoms. The experimental materials were however left in situ for more than 4 months for further observation in case some plants delayed to express the symptoms.

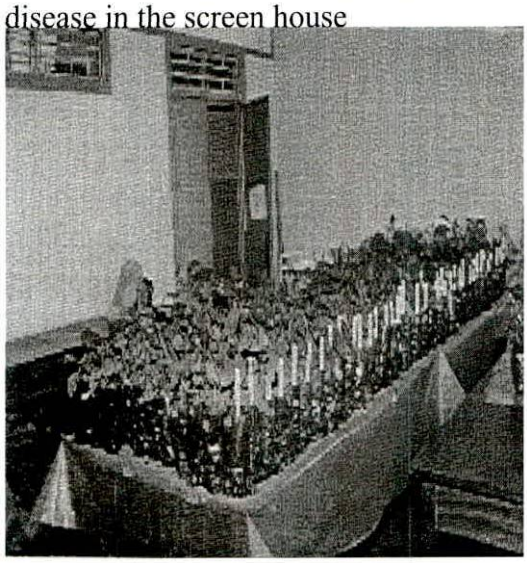
Plants that survived the first bout of inoculation were re-inoculated and incubated following procedures in the first bout. The re-inoculated plants were also monitored for symptoms and assessed for disease progress. Assessment was terminated when the plants stopped dying.

Plants that survived the re-inoculation were planted in a mother garden to generate vegetative propagules for further wilt assessment in the screenhouse and field trials in wilt 'hot spot' areas/disease nurseries.

Results

Results of the first inoculation are shown in Table 1. Plate 1 shows part of the responses of the survivor plants

Plate 2: Survivor seedlings of robusta coffee infested with coffee wilt disease in the screen house



before they were re-inoculated 214 (15.4%) seedlings out of the 1394 inoculated survived and they were healthy looking at termination of data recording after the first inoculation. Even the survivor seedlings initially showed minor wilt symptoms but later recovered and resumed healthy growth. The survivors belong to 79 (62.2%) of the 127 family progenies inoculated but the number of survivors varied between families.

These results show that 1180 (84.6%) seedlings among the open pollinated progenies of all the cultivars were susceptible to the coffee wilt disease and the 214 (15.4%) survivor seedlings belonging to 79 cultivars are resistant to coffee wilt disease. The number of resistant seedlings varies between cultivars revealing possibility of quantitative resistance to the coffee wilt disease among robusta coffee populations. The number of resistant seedlings was higher among families of collections from wilt hot spot areas than families from collections in the germplasm at CORI.

Results of re-inoculated survivors are shown in Table 2 and Figure 1. These results show that successive inoculation of survivor coffee plants can lead to the survivors succumbing to wilt disease. 63 (53%)

Table 1: Response of robusta coffee populations to Fusarium xylarioides infection under screen house condition in Uganda

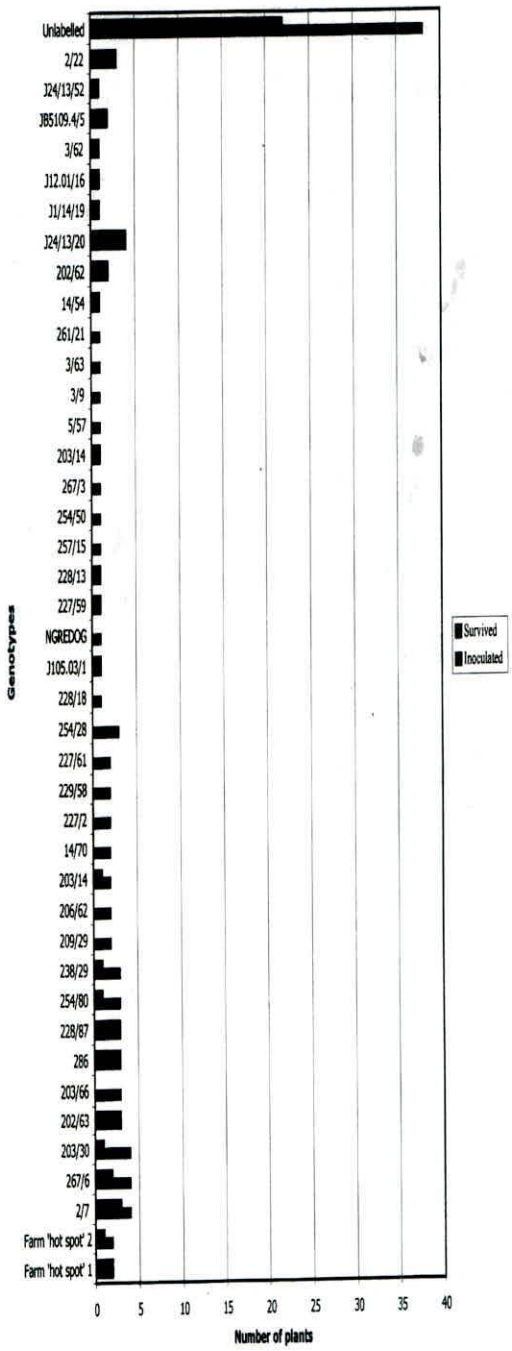
Variety	Plants		
	Inoculated	Survived	%
1. J56/20/57	12	0	0
2. J56/20/67	12	0	0
3. J94/2/22	12	3	25
4. J56/20/16	12	0	0
5. 3/71	12	2	17
6. J1/15/4	12	3	25
7. J1/14/51	10	0	0
8. J1/14/16	12	0	0
9. J1/14/19	12	4	33
10. 3/66	12	1	8
11. JB5109.4/5	12	2	17
12. J24/13/67	12	0	0
13. 3/56	12	2	17
14. 1/11	12	3	25
15. 3/20	12	1	8
16. 3/62	12	2	17
17. 14/50	12	4	33
18. 1^{s}/3 (controll)	12	0	0
19. J56/20/5	12	2	17
20. 2/67	12	0	0
21. JB5109.4/2	12	0	0
22. J12.01/16	12	2	17
23. J24/13/52	12	1	8
24. 14/1	12	0	0
25. 2/22	12	5	42
26. J94/2/64	12	2	17
27. J24/13/1	12	0	0
28. J72.01/10	12	0	0
29. 2/11	12	0	0
30. 1/12	12	1	8
31. 4/6	12	1	8
32. J94/2/18	12	2	17
33. 1/17	12	2	17
34. 3/15	12	3	25
35. 1/71	12	0	0
36. 1/15	12	1	8
37. 1/70	12	0	0
38. 2/13	12	2	17
39.257/53 (control)	12	0	0
40. 3/59	12	1	8
41. 3/49	12	1	8
42. J24/13/20	12	6	50
43. 203/66	12	0	0
44. J94/2/13	12	0	0
45 14/54	12	1	8
46. 223/32	12	0	0
47. 267^{s}/3	11	1	9
48. 261^{s}/21	12	1	8
49. 223/38	12	0	0
50. 228/87	11	5	45
51. 14/70	12	2	17
52. 261^{s}/2	12	1	8
53. 229/58	12	3	25
54. 228/12	12	1	8
55. 14/62	12	1	8
56. 3/63	12	1	8
57. 2/57	11	1	9
58. 2/7	12	6	50
59. 209/29	12	2	17
60. 202/62	12	7	58
61. 267/15	12	1	8
62. 14/60	12	0	0
63. 228	12	3	25
64. 238/29	12	3	25
65. 203/30	12	8	67
66. 228/18	12	1	8
67. 254/80	12	7	58
68. J105.03/1	12	2	17
69. 3/9	12	1	8
70. 227/2	12	4	33
71. 203/14	12	3	25
72. 203/32	12	1	8
73. 222/65	12	0	0
74. 254/28	12	6	50
75. 227/61	12	3	25
76. J1/14/21	12	0	0
77. 222/59	12	1	8
78 NGREDOG	12	1	8
79. 286	12	4	33
80. 203/62	12	7	58
81. 267^{s}/6	12	8	67
82. 222/65	12	0	0
83. 267/21	15	4	27
84. 234/37	15	0	0
85. 1/5	15	2	13
86. 4/1	15	5	33
87. J24/13/22	15	1	7
88. J24/13/5	15	2	13
89. J24/43/67	15	1	7
90. JB5109.4/3	15	0	0
91. 1^{S}/3 (control)	12	1	8
92. 3/54	12	0	0
93. 254/64	12	1	8
94. 218/52	12	0	0
95. 14/5	4	0	0
96. 1/48	4	1	25
97. J1/15/9	3	0	0
98. 245/25	3	0	0
99. 14/16	3	0	0
100. 228/57	3	0	0
101. 238/28	2	0	0
102. 222/65	10	0	0
103. 227/54	4	0	0
104. 1^{S}/6 *(control)*	12	1	8
105. 245/62	11	0	0
106. 227/58	12	0	0
107.223/32(control	8	0	0
108. 226/11	7	0	0
109. 1^{S}/2 (control)	7	0	0
110. 258/58	6	0	0
111. 223/58	11	2	18
112. 261/6	3	2	67
113. 228/15	9	0	0
114. J24/13/12	2	0	0
115. 209/139	7	0	0
116. J1/14/16	4	1	25
117. 261/15	12	1	8
118. J24/13/59	12	8	67
119. 14/56	20	0	0
120. Ka/ruku/9701/1s	9	4	44
121. Na/muk/9701/1s	4	1	25
122. Ka/ruku/9801/2s	11	0	0
123. Ka/ruku/9801/3s	9	2	22
124. Ka/ruku/9801/1s	6	1	17
125. Ka/mub/9801/2s	23	16	70
126. Ka/mub/9801/3s	11	0	0
127. Ka/mub/9801/1	10	3	30
Total	1394	214	154

N.B: Lines 120-127 are collections from wilt hot spot areas.

Table 2: Relative survival of open pollinated seedlings of robusta coffee re-infected with F. xylarioides after second inoculation

Variety	Plants		
	Re-inoculated	Survived	%
Ka/ruku/9701/1s	2	2	100
Na/muk/9701/1s	2	1	50
2/7	4	3	75
267^{s}/6	4	2	50
203/30	4	1	25
202/63	3	3	100
203/66	3	0	0
286	3	3	100
228/87	3	3	100
254/80	3	1	33
238/29	3	1	33
209/29	2	0	0
206/62	2	0	0
203/14	2	1	50
14/70	2	0	0
227/2	2	0	0
229/58	2	0	0
227/61	2	0	0
254/28	3	0	0
228/18	1	0	0
J105.03/1	1	1	100
NGREDOG	1	0	0
227/59	1	1	100
228/13	1	1	100
257/15	1	0	0
254/50	1	0	0
267^{s}/3	1	0	0
203/14	1	1	100
5/57	1	0	0
3/9	1	0	0
3/63	1	0	0
261^{s}/21	1	0	0
14/54	1	1	100
202/62	2	2	100
J24/13/20	4	4	100
J1/14/19	1	1	100
J12.01/16	1	1	100
3/62	1	1	100
JB5109.4/5	2	2	100
J24/13/52	1	1	100
2/22	3	3	100
*Unlabelled	38	22	58
Total	**118**	**63**	**53.4**

Figure 1: Relative survival of robusta coffee genotypes after a second bout of infection with xylarioides



seedlings out of the 118 survivors of the first round infection that were re-inoculated survived and continued to grow normally. Unlike in the first round, survivors of the second inoculation did not express any known wilt symptoms. Seedlings that showed wilt symptoms eventually died. The percentage of survivors in the second bout of inoculation also varied between families from 25-100%. Survivors of the second infection were planted in mother gardens to raise vegetative propagules for further studies (screenhouse and field). These plants will also be the source of resistance for improving other genotypes including the current commercial and other elite germplasm through genetic manipulation.

Discussion

At the inception of this study, the response to coffee wilt disease among the Coffee canephora populations in Uganda was not known. In lieu of such information, the number of seedlings inoculated for each cultivar was deliberately small so as to maximize on the number of families tested. Nevertheless, the results usefully revealed presence of resistance to the coffee wilt disease among the robusta coffee populations. Seedlings that survived the second round of inoculation demonstrated their resistance to the wilt disease. Certainty to the resistance will be enhanced if the survivor seedlings are subjected to infection under field conditions. The propagules (clones) thus generated from the survivors will be used for further screening in the screen house and in field coffee wilt disease nurseries to ascertain the resistance to the disease and also assess other attributes of the varieties such as yield, quality and resistance to other disease. Resistant, good quality and high yielding lines will be adopted for replanting in wilt infested areas and for expansion. It is anticipated that the replanting and expansion programmes will revive the coffee cultivation thus incomes and livelihood of farmers whose coffee has been devastated by the wilt disease. Secondly a repeat of the screening using large family sizes, particularly families that had resistant seedlings among the progenies is vital. The progenies should be screened along side their clonal parents to give a better insight of the genetic behaviour of the resistance.

Apart from varieties coded starting with J, which were imported from Indonesia in the early 1900s, the rest of the cultivars are indigenous.

Conclusion

There is resistance to coffee wilt disease among indigenous robusta coffee populations both in the genebanks at the Coffee Research institute and farms especially among survivors in wilt devastated gardens. The resistance to coffee wilt disease also exists among exotic germplasm imported from Indonesia and being conserved at CORI. The resistant genotypes have the potential for adoption as vegetatively propagated lines and for breeding to improve on the CWD resistance of current commercial robusta coffee lines. Adoption of wilt resistant varieties that are high yielding, have good cup and seed quality and resistance to other important coffee diseases will reverse effects of the wilt disease on robusta coffee production and livelihoods of its producers in Uganda.

Acknowledgement

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