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Attempts to Improve the Method for Screening Cowpea Germplasm for Resistance to Cucumber Mosaic Virus and Blackeye Cowpea Mosaic Virus

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Abstract: Use of visual symptom screening of cowpea plants in field plots improved screening for Blackeye Cowpea Mosaic Virus (BlCMV)-resistance. However, the method failed to improve the speed or accuracy of screening for Cucumber Mosaic Virus (CMV)-resistance. Plants that displayed few visual virus symptoms were selected for screening by a previously published method. This method involved screening by mechanical virus inoculation in the greenhouse. Plants having a low infection percentage in the greenhouse as judged by Direct-Antigen Coating Enzyme-linked Immunosorbent Assay (DAC-ELISA) were then screened in the field by randomized virus spread tests from inoculated spreader rows. Infection rates in these tests were also determined by DAC-ELISA. The test resulted in the detection of eleven newly discovered sources of resistance to BlCMV, but no significant new sources of CMV-resistance were found.

Key words: Resistance screening, cowpea stunt disease, cowpea, RT-PCR

INTRODUCTION

Cucumber Mosaic Virus (CMV) and Blackeye Cowpea Mosaic Virus (BlCMV) are endemic to the cowpea growing areas of the United States and other areas of the world. Alone, these viruses cause mild to moderate symptoms on cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*. These viruses interact synergistically in cowpea to cause cowpea stunt disease, which causes significant losses in cowpea and is the most serious disease of cowpea in the United States (Anderson *et al*., 1994; Pio-Ribeiro *et al*., 1978). Both viruses are aphid and seed transmitted and cowpea stunt may limit seed production in highly susceptible cowpea lines. The viruses pose a problem for distribution of germplasm and until the resistant line, GC-86L-98, was registered there were no reliable sources of resistance to CMV in cowpea (Anderson *et al*., 1996; Gillaspie 2001, 2002; Kuhn *et al*., 1966; McWilliams, 1998).

Utilizing GC-86L-98, for comparison, a prescreening and field screening method was devised for detecting cowpea stunt resistance (Gillaspie, 2006). This method relied on choosing cowpea lines at random to test for resistance. The cowpea germplasm program at the Plant Genetic Resources Conservation Unit of the National Plant Germplasm System at Griffin, GA, routinely performs regenerations of lines in the germplasm collection that have become depleted through distribution or have reduced viability because of age. These regenerations are done in the field every summer and we have observed that some lines seem to have few virus symptoms while others express severe symptoms.

The test described here shows what happens when those germplasm lines which express few symptoms in field tests are subjected to the screening method described by Gillaspie (2006). The purpose was to determine if visual symptoms of these field grown plants would be useful for the initial stage of resistance screening.

MATERIALS AND METHODS

Plants for this test were chosen from among the 200 lines that were regenerated at Griffin, GA, in field plots. Notes were made as the season progressed on the presence or absence of virus symptoms in these lines. No determination was made concerning the viruses that were present in each line, but there was a determination made by Direct-Antigen-coating Enzyme-Linked-Immunosorbent Assay (DAC-ELISA) of the viruses present in the field (Gillaspie *et al*., 1995). Lines with few or no visible symptoms were then chosen for further testing.

Fifteen seed of each of the cowpea lines to be tested were planted in the greenhouse in 10 cm pots (5 seeds pot) for each virus to be tested. Seeds of cowpea lines Coronet (susceptible control) and GC-86L-98 (resistant control) were included with each test. When the plants were at the fully expanded cotyledon-leaf stage they were mechanically inoculated with either CMV or BlCMV as previously described (Gillaspie, 2006). The plants were reinoculated mechanically one week after the first inoculation to prevent escapes. At the third-trifoliolate leaf stage a leaf was taken from each plant and tested by DAC-ELISA for the presence of the virus.

Cowpea lines with a low percentage of infection were then selected for the field resistance screening as reported by Gillaspie (2006).

Seed of the eleven lines chosen for the field testing as well as seed of Coronet and GC-86L-98 were direct-seeded into 3 m plots with 3 m between plots and 3 m between rows. Plots were arranged in a randomized block design with rows of mechanically inoculated Coronet plants transplanted from the greenhouse into plots between the test plots so, that each test plot row was next to a row of plants which had been mechanically inoculated. The infected plants were transplanted into the field when the test plants were at the second-to-third trifoliolate leaf stage. This meant that the test plants and the transplanted spreader-row plants were about the same size. The test plants were sampled after one and two months of exposure to the virus from the infected spreader row plants. A leaf was taken from the newly expanded leaves of each plant and tested by DAC-ELISA.

RESULTS AND DISCUSSION

A total of 73 lines were chosen to be tested in the greenhouse based on the lack of visible virus symptoms in these lines in field plots. The first batch of 24 lines had much lower than normal infection with CMV as judged by the infection level on Coronet but the infection percentage for BlCMV on Coronet was about as would be expected (Table 1). The infection percentages on Coronet with CMV in the other two tests were higher than with test 1 (Table 2 and 3), but the rates were still lower than were obtained with the same inoculum a year earlier. The freeze dried tissue used for this inoculum seems to have lost some viability. The rates of infection for BlCMV in these last two tests were as high as expected for previous studies. Based on these data, 11 lines were chosen to be screened further in field virus spread tests. These selections were made considering reactions to both viruses so that those lines with a low percentage of infection with both viruses in the greenhouse were selected for the field.

Table 4 shows that results of the field virus spread test. Interestingly, the 11 lines were all resistant to BlCMV as they all had lower infection percentages than the susceptible control (Coronet) and were not significantly different from the resistant control (GC-86L-98). Only PIs 581001, 581104 and 583174 were as low as the resistant control for CMV resistance in the July assay, but these were not resistant enough to have low infection percentages in August. Note that the lines varied from July to August in their CMV resistance levels

Table 1: Greenhouse screening for Cucumber Mosaic Virus (CMV) and Blackeye Cowpea Mosaic Virus (BlCMV) resistance in cowpea lines determined by direct-antigen-coating-enzyme-linked immunosorbent assay (DAC-ELISA) (March 2006)

	CMV-infected		BlCMV-infected	
Cowpea line	Plants/total plants	Infected (%)	Plants/total plants	Infected (%)
PI 352941	0/13	0	8/11	73
PI 353319	2/15	13	7/13	54
PI 354500	0/11	0	3/6	50
PI 354580	0/10	0	0/12	0
PI 354729	0/10	0	0/9	0
PI 354823	0/10	0	8/10	80
PI 353074	0/14	0	7/13	54
PI 419165	2/10	20	0/13	0
PI 578898	0/14	0	8/12	67
PI 578911	2/14	14	4/12	33
PI 292570	2/15	13	0/14	0
PI 339638	1/11	9	9/13	69
PI 580998	0/15	0	0/14	0
PI 581001	1/12	8	0/12	0
PI 581004	0/13	0	2/11	18
PI 581014	0/15	0	7/14	50
PI 581017	0/15	0	8/14	57
PI 581019	2/12	17	10/14	71
PI 581056	0/13	0	12/14	86
PI 581059	0/14	0	11/13	85
PI 581061	0/9	0	11/13	85
PI 581065	2/12	17	12/14	86
PI 581069	2/13	15	11/14	79
PI 581070	2/14	14	11/11	100
Coronet	5/13	38	8/15	53
GC-86L-98	1/15	7	0/15	0

Table 2: Greenhouse screening for Cucumber Mosaic Virus (CMV) and Blackeye Cowpea Mosaic Virus (BlCMV) resistance in cowpea lines determined by direct-antigen-coating-enzyme-linked immunosorbent assay (DAC-ELISA) (early April 2006)

	CMV-infected		BlCMV-infected	
Cowpea line	Plants/total plants	Infected (%)	plants/total plants	Infected (%)
PI 581079	1/13	8	12/12	100
PI 581083	1/15	7	0/14	0
PI 581084	1/10	10	11/11	100
PI 581086	2/13	15	10/10	100
PI 581089	8/12	67	10/10	100
PI 581091	4/11	36	0/10	0
PI 581098	1/11	9	10/11	91
PI 581100	11/12	92	13/14	93
PI 581104	0/13	0	1/15	7
PI 581105	2/8	25	0/12	0
PI 581106	3/14	21	5/8	63
PI 581107	1/10	10	12/14	86
PI 581109	5/13	38	11/12	92
PI 581113	2/11	18	2/14	14
PI 581119	2/9	22	14/14	100
PI 581120	6/13	46	12/14	86
PI 581132	1/12	8	11/12	92
PI 581133	3/11	27	13/13	100
PI 581134	5/13	38	12/12	100
PI 581135	6/10	60	10/10	100
PI 581141	3/12	25	0/12	0
PI 581142	3/11	27	10/10	100
PI 581144	4/10	40	14/14	100
PI 581146	3/5	60	2/5	40
PI 581148	2/12	17	12/12	100
Coronet	8/15	53	12/14	86
GC-86L-98	1/14	7	0/14	0

Table 3: Greenhouse screening for Cucumber Mosaic Virus (CMV) and Blackeye Cowpea Mosaic Virus (BlCMV) resistance in cowpea lines determined by direct-antigen-coating-enzyme-linked immunosorbent assay (DAC-ELISA) (mid April 2006)

	CMV-infected		BlCMV-infected	
Cowpea line	Plants/total plants	Infected (%)	Plants/total plants	Infected (%)
PI 581151	6/13	46	12/12	100
PI 581152	4/10	40	9/10	90
PI 581156	7/10	70	3/12	25
PI 581157	3/15	20	9/14	64
PI 581159	3/12	25	13/13	100
PI 581160	6/11	55	13/13	100
PI 581162	8/13	62	12/12	100
PI 581163	11/14	71	14/15	93
PI 582417	3/14	21	14/15	93
PI 582793	4/14	29	15/15	100
PI 582922	3/12	25	5/11	45
PI 582932	1/10	10	11/13	85
PI 582941	10/14	71	0/15	0
PI 582942	4/13	31	13/13	100
PI 582943	8/14	57	0/15	0
PI 582959	7/15	47	15/15	100
PI 582965	2/15	13	9/14	64
PI 582970	7/14	50	6/14	43
PI 583077	0/12	0	8/13	62
PI 583100	5/15	33	2/14	14
PI 583102	6/15	40	8/14	57
PI 583174	0/10	0	0/12	0
PI 583182	8/15	53	0/15	0
PI 583494	4/15	27	15/15	100
Coronet	10/14	71	12/13	92
GC-86L-98	3/14	21	0/15	0

Table 4: Field screening test for Cucumber Mosaic Virus (CMV) and Blackeye Cowpea Mosaic Virus (BlCMV) resistance for cowpea lines using virus-spread from infected rows of susceptible cowpeas and detection by direct antigen coating-enzyme-linked immunosorbent assay (2006)[y]

	Infected/total plants (mean% infected)			
	July		August	
Cowpea line	CMVz	BlCMV[z]	CMVz	BlCMV[z]
PI354580	17/34(41)bcde	0/34(0)b	9/15(60)abc	0/15(0) b
PI354729	7/10(42)abc	0/10(0)b	5/12(45)bc	0/12(0)b
PI419165	22/30(69)abcd	0/30(0)b	16/19(87)ab	0/19(0b
PI580998	15/21(74)abc	0/21(0)b	12/12(100)a	0/12(0)b
PI581001	8/22(32)cde	1/22(11)b	17/21(84)ab	0/21(0)b
PI581004	21/24(86)a	0/24(0)b	20/22(88)ab	3/22(9)b
PI581083	9/20(42)abcde	0/20(0)b	11/14(75)abc	0/14(0)b
PI581104	6/19(18)e	0/19(0)b	13/16(84)ab	0/16(0)b
PI581105	26/34(77)ab	0/34(0)b	16/18(89)ab	0/18(0)b
PI581141	15/23(77)ab	2/23(5)b	14/17(73)abc	5/17(24)b
PI583174	7/22(27)de	0/22(0)b	12/13(95)a	1/13(5)b
Coronet	23/27(72)abc	13/27(36)a	12/15(58)abc	11/15(55)a
GC-86L-98	6/16(36)bcde	0/16(0)b	6/15(34)c	0/15(0)b

[y]Test seeds were planted in the field and Coronet seeds were planted in the greenhouse on the same day. Infected Coronet plants were transplanted into the field plots to serve as inoculum when the test plants were at the first or second trifoliolate leaf stage. Test was planted in a randomized block design with three replications, [z]Means in each column with the same letter(s) are not significantly different at Alpha = 0.05

Table 5: Analysis of variance for effects of Cucumber mosaic virus and Blackeye cowpea mosaic virus on cowpeas in field test in 2006

Source	df	Ms	F	p-value
Rep	2	1175.5833	2.44	0.0914
Month	1	4719.0000	9.81	0.0022
Virus	1	139680.9231	290.31	<0001
Cowpea line	12	1314.6368	2.73	0.0028
Month-virus	1	2448.2308	5.09	0.0260
Month-cowpea	12	555.1667	1.15	0.3250
Virus-cowpea	12	1146.4786	2.38	0.0088
Residual	114	481.1427	…	…

relative to each other. The ANOVA analysis described in between the viruses (Table 5).

Utilizing data from one year's observation, it can be concluded that the presence or absence of visual symptoms in a field test is not enough to select for resistance candidates for CMV. The CMV-symptoms are apparently too mild in many cowpea genotypes for accurate diagnosis. This study identified a number of lines as possible BlCMV-resistant genotypes. In fact, if one can rely on the results from the greenhouse- portion of the test, there may have been a few more than the eleven in the field test. These lines would provide sources of genes to study for development of cowpea stunt resistant cultivars. However, the number of CMV-resistant genotypes is still limited and it is assumed that the best stunt resistance would come from combining genes from lines with resistance to both viruses.

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