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Assessing Yield Potential of Cowpea Genotypes Grown Under Virus Pressure

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Abstract. Cowpea or Southernpea [*Vigna unguiculata* (L.) Walp.] is an important grain legume in many parts of the tropics. However, viral diseases, particularly *Cucumber mosaic virus* (CMV) and *Blackeye cowpea mosaic virus* (BICMV), can be a limiting factor in cowpea production. We evaluated in replicated field plots and under virus pressure nine PIs (441919, 441925, 441917, 147071, 146618, 180014, 180355, 194208, 612607) and three commercial cultivars (Coronet, KnuckleHull-VNR, Pinkeye Purplehull), some of which had shown absence of symptoms for CMV and BICMV in unreplicated, seed regeneration plots of the U.S. cowpea collection. Only 3% of all plots had plants infected with both CMV and BICMV in 2003 and 2004. This percentage increased to 47% in 2005. The accession PI 441917 had the highest 3-year mean for grain yield. However, PI 147071, PI 180014, and 'KnuckleHull-VNR' had higher seed protein concentration than other genotypes, but their grain yield was significantly lower than that of PI 441917. The cultivar Coronet and PI 180355 attained midbloom and maturity earlier than the other genotypes. Overall, PI 441917 outperformed all other genotypes for grain yield, including virus-resistant PI 612607 and the cultivar KnuckleHull-VNR. This accession is in the process of being released as a virus-tolerant genotype and should be useful in cowpea breeding programs to help control yield losses by CMV and BICMV.

Cowpea or Southernpea [*Vigna unguiculata* (L.) Walp] is the most economically important indigenous African legume crop (Langyintuo et al., 2003). The crop is also an important staple in many parts of the United States, South and Central America, the Caribbean, India, and Australia (Quinn and Myers, 2002). Worldwide production of cowpea is over 8 million hectares.

Viral diseases are a limiting factor in cowpea production and hence, identifying sources of resistance is an important objective of cowpea breeding programs (Ehlers and Hall, 1997; Singh, et al., 2003). Several improved cowpea varieties with resistance to *Cowpea yellow mosaic virus* and *Cowpea aphid borne mosaic virus* have been released for African growers by the International Institute of Tropical Agriculture (Asafo-

Adjei, et al., 2005; Mligo and Singh, 2007; Touré and Singh, 2005). Two of the most common viruses affecting cowpea production in the United States are the *comovirus Cucumber mosaic virus* (CMV) and the potyvirus *Blackeye cowpea mosaic virus* (BICMV). BICMV is regarded as a distinct strain of *Bean common mosaic virus* (ICTV, 2002). The accession GC-86L-98 (PI 612607) is a germplasm line with resistance

to CMV and high resistance to BICMV released in 2001 (Gillaspie, 2002). However, few cultivars with CMV or BICMV resistance are available to growers in the United States. The cultivar KnuckleHull-VNR, a crowder-type cowpea with resistance to BICMV and root-knot nematodes, was released in 2002 by Fery et al. (2004). After a 3-year field testing period, this cultivar showed high resistance to BICMV and average yield of 916 kg·ha⁻¹. For years, seed of photoperiod-sensitive accessions in the U.S. Department of Agriculture (USDA) cowpea germplasm collection conserved at Griffin, GA, has been regenerated at the USDA-ARS facilities in Isabela, PR (Gillaspie et al., 1999). Genetic diversity for tolerance to viral diseases has been observed by the authors during these regeneration cycles. Tolerance is defined in this study as the ability of a genotype to produce a good crop even when it is infected with a pathogen (Agrios, 2005).

The present study was conducted with the objective of determining yield potential and seed protein of various cowpea genotypes that have shown viral disease tolerance in unreplicated seed regeneration plots as an effort to identify materials that could be used by breeders in cowpea improvement programs.

Materials and Methods

The study was conducted at the USDA-ARS Research Farm in Isabela, PR. The Coto soil is a well-drained Oxisol (clayey, kaolinitic, isohyperthermic, Typic Hapludox). Relevant soil chemical properties for evaluation plots during each year are presented in Table 1. The 24-year mean annual rainfall is 1649 mm, and Class A pan evaporation is 1672 mm. Mean monthly maximum and minimum air temperatures are 29.8 and 19.9 °C. Weather data during the experimental period are presented in Table 2.

Field experiments were established on 10 June 2003, 10 Feb. 2004, and 24 Feb. 2005 using PIs 146618, 147071, 180014, 180355,

Table 1. Average preplant soil characteristics at three test plots in Isabela, PR, measured to a depth of 30.5 cm.

Soil characteristic	Year		
	2003	2004	2005
pH in water	8.0	7.4	6.4
pH in calcium chloride	7.5	7.0	6.0
Ammonium nitrogen (mg·kg ⁻¹)	12	17	16
Nitrate nitrogen (mg·kg ⁻¹)	8	8	8
Organic C (%)	0.43	0.56	0.44
P (mg·kg ⁻¹)	47	18	42
K (mg·kg ⁻¹)	302	240	247
Ca (mg·kg ⁻¹)	4077	1830	1005
Mg (mg·kg ⁻¹)	184	81	82

Table 2. Average rainfall, maximum and minimum air temperatures, and Class A pan evaporation in Isabela, PR, during the experimental period.

	Rainfall (mm)	Temperature (°C)		Pan evaporation (mm)
		Maximum	Minimum	
2003	139.2	31.4	22.7	151.9
2004	115.3	28.6	20.0	150.1
2005	106.7	29.2	20.3	143.5

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Table 3. Phenotypic descriptors² of 12 cowpea genotypes grown in Isabela, PR.

Genotype	Flower color	Leaf shape	Plant habit	Dry pod color	Pod placement	Pod position	Pods per peduncle	Seed coat color	Seed pattern color	Seed pattern	Seed shape	Seed size	Seed texture
PI 441919	L	SH	P	S	BF	I	2	T	T	S	C	V	S
PI 441925	L&W	SH	P	S	FL	P	2	W	B	E	N	M	M
PI 441917	L	H	P	S	BF	P	2	T	Bl	SP	S	L	S
PI 147071	L	H	P	S	FL	P	2	R	R	S	N	L	S
PI 146618	W	H	P	S	BF	P	2	M	B	H	N	M	S
PI 180014	L	SH	P	S	FL	P	3	B	B	S	S	M	M
PI 180355	P	SH	SP	B	FL	I	3	B	B	S	N	S	S
PI 194208	L	H	E	S	FL	E	3	B	B	S	S	M	S
PI 612607	W	SH	P	S	FL	P	2	W	B	E	N	L	R
Pinkeye Purplehull	W	SH	SP	P	AF	P	3	W	P	E	N	M	R
Coronet	W	H	SP	P	FL	P	2	W	P	E	N	M	M
KnuckleHull-VNR	L	SH	SP	P	FL	P	2	T	T	S	N	M	S

²Descriptor coding: Flower color: L (Lavender), W (white), P (purple); Leaf shape: H (hastate), SH (subhastate); Plant habit: E (erect), SP (semiprostrate), P (prostrate); Dry pod color: S (straw), P (purple), B (brown); Pod placement: AF (above foliage), FL (foliage level), BF (below foliage); Pod position: E (erect), I (intermediate), P (pendant); Pods per peduncle: actual number; Seed coat color: B (brown), M (mixed), R (red), T (tan), W (white/cream); Seed pattern color: Bl (black), B (brown), P (purple), R (red), T (tan); Seed pattern: E (eye), H (Holstein), S (solid), SP (speckled); Seed shape: C (crowder, seed flat on both ends), S (semicrowder, seed flat on one end and rounded on other), N (noncrowder, seed rounded on both ends); Seed size: L (large), M (medium), S (small), V (variable); Seed texture: R (rough), S (smooth), M (mixed).

194208, 441917, 441919, 441925, and 612607 and the commercial cultivars Coronet, KnuckleHull-VNR, and Pinkeye Purplehull. The accession PI 612607 was included in the study as a virus-resistant control for CMV and BICMV and 'KnuckleHull-VNR' as a BICMV-resistant cultivar (Fery et al., 2004; Gillaspie, 2006). The cultivars Coronet and Pinkeye Purplehull were used as susceptible controls to both viruses (Gillaspie, 2002). Phenotypic descriptors of genotypes are shown in Table 3. The experiments were planted in a randomized complete block design with three replications. Because of the prostrate growth habit of some genotypes, experimental plots consisted of 3-m rows spaced 6.1 m apart. Seedlings were thinned to an in-row spacing of 15 cm. Fertilization was provided 1 month after planting using a 16N-1.7P-3.3K commercial mixture at a rate of 132 kg-ha⁻¹. Plants were drip irrigated as necessary.

Viral infection of plants in the 2003 experiment occurred naturally through insect vectors, mainly aphids. In 2004 and 2005, however, all experimental plants were mechanically inoculated ≈3 weeks after planting with extracts from leaf tissue obtained from symptomatic plants of the cultivar 'Coronet'. This tissue was previously determined to be infected with BICMV and CMV by direct antigen-coated enzyme-linked immunosorbent assay (DAC-ELISA) as described by Gillaspie et al. (1995). Inoculum was prepared from trifoliate leaves of the cultivar Coronet that were excised from plants and macerated in 1% potassium phosphate buffer, pH 7.2 (1 g fresh tissue with 10 mL buffer). Experimental plants were then inoculated by dusting 320-grit Carborundum onto two trifoliate leaves and applying the inoculum with a cotton swab. Inoculation was repeated 2 weeks after the first inoculation to reduce the possibility of plants escaping infection. Leaf samples were taken from eight random plants per row at 57 d after planting (DAP) in 2003, 62 DAP in 2004, and 57 DAP in 2005 and sent by overnight mail to

Griffin, GA, for virus testing by DAC-ELISA. These dates are close to the mid-bloom period. In all experiments, population of aphids, bean leaf beetle (*Ceratoma trifurcata*), and ants were high near flowering time and a single application of methomyl was done at that time. Midbloom and maturity were determined as the number of days after planting at which 50% of plants flowered and the first dry pod appeared, respectively. Pods were harvested by hand as they matured, dried at 38 °C, 25% relative humidity, and shelled. Plant height was measured with a ruler at midrow. Pod length was determined by averaging the length of 10 mature pods from five plants selected randomly from each plot. Random 100-seed samples from each experimental unit were weighed to determine seed weight. Seed N was determined using the micro-Kjeldahl procedure and multiplied by the factor 6.25 to obtain percent seed protein (AOAC, 2000).

Analysis of variance was carried out using the GLM procedure of SAS (release 9.1 for Windows; SAS Institute, Cary, NC). After

significant F tests at $P < 0.05$, mean separation was performed using the least significant difference.

Results and Discussion

Combined analysis of variance revealed statistically significant ($P \leq 0.001$) genotype and year effects on all parameters measured in the study (Tables 4 and 5). The genotype × year interaction was significant ($P \leq 0.001$) for grain yield, midbloom, days to maturity, plant height, and pod length.

For most genotypes, grain yield was significantly higher in 2003 than in other years. This response was probably the result of less virus incidence resulting from plants not being inoculated in 2003 (Table 4). ELISA results showed that in 2003, only 8% of all plots had plants infected with BICMV and 28% with CMV. In 2004, 44% and 8% of all plots had plants infected with BICMV and CMV, respectively, whereas in 2005, 61% and 72% of plots were infected with BICMV and CMV, respectively (Table 6).

Table 4. Grain yield of 12 cowpea genotypes grown in Isabela, PR, during 2003 to 2005.

Genotype	3-yr	Grain yield (kg-ha ⁻¹)		
		2003	2004	2005
441919	826.7	1436.3	780.7	263.0
441925	558.6	957.1	418.5	300.2
441917	1420.2	1902.8	1436.6	921.1
147071	686.9	1228.7	566.9	265.2
146618	365.1	587.9	343.4	164.1
180014	1033.4	1554.6	1182.4	363.1
180355	316.2	579.3	201.3	168.1
194208	496.8	710.8	321.3	457.9
612607	1247.6	1451.6	1612.0	679.4
Pinkeye Purplehull	449.7	706.7	447.4	195.0
Coronet	578.2	970.7	566.4	197.6
KnuckleHull-VNR	550.4	897.2	473.4	280.7
Average	710.8	1082.0	695.9	354.6
LSD (0.05) ²	152.7	269.6	315.1	210.0
Genotype	***	***	***	***
Year (Y)	***	—	—	—
G × Y	***	—	—	—

²Least significant difference at $P = 0.05$.

***Significant at $P \leq 0.001$.

Table 5. Average seed protein, weight of 100 seed, midbloom, maturity, plant height, and pod length of 12 cowpea genotypes in field experiments conducted at Isabela, PR, in 2003, 2004, and 2005.

Genotype	Seed protein (%)	100-seed wt (g)	Midbloom (d)	Maturity (d)	Plant ht (cm)	Pod length (cm)
PI 441919	23.4	17.0	55.3	74.3	49.4	21.1
PI 441925	23.9	11.7	49.9	66.9	45.8	17.6
PI 441917	22.1	20.1	46.4	65.3	50.6	23.2
PI 147071	25.1	13.0	45.8	65.7	35.0	25.1
PI 146618	23.5	8.6	43.8	62.3	33.8	22.8
PI 180014	24.8	8.6	49.2	66.7	55.7	14.2
PI 180355	23.0	5.5	42.1	59.0	61.6	11.0
PI 194208	22.7	9.9	49.3	65.3	62.8	16.2
PI 612607	21.2	18.9	60.8	80.8	42.9	15.1
Pinkeye Purplehull	22.7	14.6	44.1	61.1	47.2	16.9
Coronet	22.8	14.1	42.4	59.8	47.9	16.4
KnuckleHull-VNR	24.8	14.8	44.7	62.4	52.2	17.7
Average	23.3	13.1	47.8	65.8	48.7	18.1
LSD (0.05) ^z	1.2	1.1	2.2	2.6	4.1	1.1
Genotype (G)	***	***	***	***	***	***
Year (Y)	***	***	***	***	***	*
G × Y	NS	NS	***	***	***	**

^zLeast significant difference at $P = 0.05$.

ns,***,**Nonsignificant or significant at $P \leq 0.001, 0.01, \text{ or } 0.05$, respectively.

Table 6. Absorbance readings for *Cucumber mosaic virus* (CMV) and *Blackeye cowpea mosaic virus* (BICMV) in cowpea genotypes determined by direct antigen coating–enzyme-linked immunosorbent assay in 2003, 2004, and 2005 from samples collected at approximately midbloom.

Genotype	Replication	Yr					
		2003		2004		2005	
		CMV	BICMV	CMV	BICMV	CMV	BICMV
PI 441919	1	0	0	0	0	0.281	0.347
PI 441925		0.202	0	1.268	0.109	1.536	0.774
PI 441917		0	0	0	0.116	0	0
PI 147071		0	0	0	0	0	0
PI 146618		0	0	0	0.153	1.501	0.238
PI 180014		0	0	0	0	2.814	1.197
PI 180355		0.317	1.900	0	0	1.697	1.789
PI 194208		0	0	0	0	0.449	0
PI 612607 ^z		0	0.467	0	0.130	1.265	0
Pinkeye Purplehull		0.803	0	0	0.190	1.645	0.659
Coronet		0	0	0	0.125	1.931	3.560
KnuckleHull-VNR ^y		0.161	0	0	0	0.496	0
PI 441919	2	0	0	0	0	1.710	0.345
PI 441925		0.631	0	0	0.177	1.094	0.823
PI 441917		0	0	0	0	0	0
PI 147071		0	0	0	0.145	0.599	0
PI 146618		0	0	0	0	0	0.199
PI 180014		0	0	0	0.151	0	1.459
PI 180355		0	0	0	0.126	2.252	1.444
PI 194208		0	0	0	0.150	0.630	0
PI 612607 ^z		0	0	0	0.120	0	0
Pinkeye Purplehull		0.561	0	0	0.168	2.393	0.756
Coronet		0.405	0	0	0	2.937	0.395
KnuckleHull-VNR ^y		0	0	0	0.134	0.305	0
PI 441919	3	0.787	0	0	0	0	1.155
PI 441925		0	0	0	0	1.097	1.271
PI 441917		0	0	0	0	0	0
PI 147071		0	0	0	0	0	0
PI 146618		0	0	0	0	0.144	0.549
PI 180014		0	0.963	0	0	0.619	0.907
PI 180355		0.889	0	0.731	0	0	0.957
PI 194208		0	0	0	0	2.532	0
PI 612607 ^z		0	0	0	0	0.786	0
Pinkeye Purplehull		0.710	0.143	0	0.475	1.329	0.434
Coronet		0	0	0	0	0.482	0.805
KnuckleHull-VNR ^y		0.239	0	1.683	0	0.404	0

^zCowpea genotype reported by Gillaspie (2002) to have CMV and BICMV resistance.

^yCowpea cultivar reported by Fery et al. (2004) to have BICMV resistance.

^xZero absorbance denotes absence of virus.

Only 3% of all plots had plants infected with both BICMV and CMV in 2003 and 2004. This percentage increased to 47% in 2005. BICMV and CMV interact synergistically in cowpea to cause cowpea stunt disease, which causes significant losses in

yield and is the most serious disease of cowpea in the continental United States (Anderson et al., 1996; Gillaspie, 2006; Martin et al., 2004). The synergistic interaction between BICMV and CMV was probably a major cause of the drastic yield

reduction in 2005 (Tables 4 and 5). Visual observations showed a higher incidence of leaf mottling, leaf distortion, and plant stunting in 2005. All the factors contributing to this reduced yield of all genotypes in 2005 are not known, but they may involve such things as other viruses not considered in this study, fluctuations in temperature, and timing of rainfall.

It is noteworthy that ELISA readings for leaf samples of PI 441917 demonstrated no CMV and BICMV infection except in 2004 when a low absorbance reading was obtained for BICMV. This contrasts with the susceptible controls, ‘Coronet’ and ‘Pinkeye Purplehull’, which had high absorbance values, particularly in 2005 (Table 6). PI 147071 also had low absorbance readings in most years, but mean grain yield was significantly lower than that of other genotypes (Tables 4 and 6). There were no positive absorbance readings for ‘KnuckleHull-VNR’, thus confirming the resistance of this cultivar to BICMV (Table 6). Several plots of PI 612607 were infected with CMV and BICMV, which contrasts the results obtained by Gillaspie (2002) describing this genotype as resistant to both viruses. However, in that study, plants were not inoculated and instead, plants of cultivar Coronet were mechanically inoculated with CMV and BICMV and used as spreader rows of these viruses. Therefore, PI 612607 could have field resistance to BICMV and CMV that was overcome by direct inoculation in this study. Still, this genotype had the second-best 3-year mean yield (Table 4) making it an excellent producer even when infected with these viruses.

Genotype 441917 had the highest grain yield in 2 of the 3 years and the highest 3-year mean for this variable. This genotype out-yielded the resistant control PI 612607 as well as all commercial cultivars evaluated (Table 4). Previous work (Gillaspie, 2006) had shown PIs 441917 and 441919 to have resistance to BICMV and CMV; however, grain yield was not determined in that study. Our results showed that PI 441917 is an excellent yielder even when plants were infected with BICMV or CMV (Tables 4 and 6). The mean grain yield obtained by PI 441917 in this study (Table 4) compares favorably with the mean yield ($1424 \text{ kg}\cdot\text{ha}^{-1}$) of 25 improved, early-maturing varieties grown with good management in West Africa (Singh et al., 2003). In contrast, as compared with PI 441917, PI 441919 had a significant grain yield decline in 2004 and 2005 (Table 4). Throughout the experimental period, there were no significant differences in grain yield among the three commercial cultivars (Table 4), but their yield was significantly lower than for PI 441917.

Genotypes 147071, 180014, 441925, and ‘KnuckleHull-VNR’ had a significantly higher seed protein concentration than the other genotypes (Table 5). It is noteworthy that genotypes with high yield such as PI 441917 and PI 612607 had significantly lower seed protein concentration than these genotypes.

Seed weight varied significantly among genotypes (Table 5). The accession PI 441917 had the highest 100-seed weight, whereas PI 180355 had the lowest weight. There were no significant differences in 100-seed weight between genotypes 146618 and 180014 and among 'Pinkeye Purplehull', 'Coronet', and 'KnuckleHull-VNR'; all other genotypes differed significantly for this variable.

Midbloom mean for all genotypes was 48 d (Table 5). The accessions PI 612607 and 441919 took significantly longer to attain midbloom and reach maturity than other genotypes. The cultivar Coronet and PI 180355 attained midbloom and maturity sooner than the rest of the genotypes (Table 5). The accessions PI 194208 and PI 180355 were significantly taller than the rest of the genotypes, averaging 62.2 cm in height. In contrast, PI 147071 and 146618 were significantly shorter than the rest of the genotypes. Pod size was significantly longer in PI 147071, whereas PI 180355 produced the shortest pods.

In conclusion, 12 cowpea genotypes were evaluated during 3 years for field performance under BICMV and CMV pressure. The accession PI 441917 outperformed all other genotypes for grain yield, including virus-resistant genotypes such as PI 612607 and cultivar KnuckleHull-VNR. The accession PI 441917 is in the process of being released as a virus-tolerant genotype and should be useful in breeding programs to

help control yield losses caused by BICMV, CMV, or cowpea stunt disease.

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