

FIELD PERFORMANCE OF COWPEA GENOTYPES GROWN UNDER VIRUS PRESSURE IN PUERTO RICO

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ABSTRACT

Cowpea [*Vigna unguiculata* (L.) Walp] is an important grain legume in many regions 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 under virus pressure the performance of four PI's (441917, 441919, 612607, 180014) and a commercial cultivar (Coronet), in Puerto Rico during 2007-2008. Viral infection of plants with BICMV and CMV in these experiments occurred naturally through insect vectors from mechanically inoculated spreader-row plants, as determined by direct antigen-coated enzyme-linked immunosorbent assay (DAC-ELISA). PI441917 had the highest grain yield (3682.8 kg ha⁻¹) among all genotypes except for the virus resistant control PI 612607, which was not significantly different. PI's 180014 and 612607 had the highest seed protein concentration. The cultivar Coronet attained midbloom and maturity earlier than the other genotypes. These results confirm previous studies which showed that PI 441917 is an excellent yielder when plants are grown under virus pressure. This accession should be useful in cowpea breeding programmes to help control yield losses by CMV and BICMV.

Key Words: Blackeye cowpea mosaic virus, *Vigna unguiculata*

RÉSUMÉ

La cornille [*Vigna unguiculata* (L.) Walp] est un important grain des légumineuses trouvé dans beaucoup de régions tropicales. Par ailleurs, les maladies virales particulièrement le virus de la mosaïque du concombre (CMV) et le *virus de la mosaïque de la cornille Blackeye* (BICMV), peuvent être un facteur limitant de la production de la cornille. Nous avons évalué sous pression virale les performances de quatre PI (441917, 441919, 612607, et 180014) et un cultivar commercial (Coronet), à Puerto Rico au cours des années 2007-2008. L'infection virale des plants avec BICMV et CMV dans ces essais a eu lieu naturellement à travers les vecteurs d'insectes par le "spread-row" mécanique des plantes, comme déterminé par l'enzyme-associé immunosorbant d'essai antigène enrobé (DAC-ELISA). PI441917 avait le rendement en grain le plus élevé (3682.8 kg ha⁻¹) parmi tous les génotypes, excepté pour le virus témoins résistant PI 612607, qui n'était pas significativement différent. PI 180014 et 612607 avaient une concentration élevée en protéines. Le cultivar Coronet avait atteint la mi-floraison et la maturité plus tôt que d'autres génotypes. Ces résultats confirment ceux des études antérieures par lesquelles PI 441917 est un excellent produit lorsque les plants croissent sous pression virale. Cette accession pourrait être utile dans des programmes d'amélioration de la cornille pour aider à contrôler les pertes de rendement par CMV et BICMV.

Mots Clés: Virus de la mosaïque de la cornille Blackeye, *Vigna unguiculata*

INTRODUCTION

Cowpea or Southernpea [*Vigna unguiculata* (L.) Walp.] is a nutritious annual legume and an important staple in many parts of the world (Quinn and Myers, 2002; Langyintuo *et al.*, 2003). Since 2004, the area for cowpea production worldwide has increased by 34% to almost 12 million hectares in 2008 (FAO, 2008; 2010).

Viral diseases are a limiting factor in cowpea production worldwide. Hence, identifying sources of resistance is an important objective of cowpea breeding programmes (Ehlers and Hall, 1997; Singh *et al.*, 2003). In 2004, the U.S.A. *Vigna* Crop Germplasm Committee (2004) revised its list of priority evaluation needs for cowpea to include resistance against important cowpea viruses. Developing disease resistant cowpea genotypes is also a major objective of the Bean/Cowpea Collaborative Research Support Programme funded by the U.S. Agency for International Development (Hall *et al.*, 2003).

Two of the most common viruses affecting cowpea production in the United States are the cucumovirus, *Cucumber mosaic virus* (CMV), and the potyvirus, *Blackeye cowpea mosaic virus* (BICMV). BICMV is regarded as a distinct strain of the *Bean common mosaic virus* (ICTV, 2010). The accession GC-86L-98 (PI-612607) is a germplasm line with resistance to CMV and to BICMV that was released in 2001 (Gillaspie, 2002). However, few cultivars with CMV or BICMV resistance are currently available to growers worldwide. The cultivar, KnuckleHull-VNR, a crowder-type of cowpea with resistance to BICMV and root-knot nematode, was released in 2002 by Fery *et al.* (2004). After a 3-year field testing period, this cultivar showed high resistance to BICMV and an average yield of 916 kg ha⁻¹ (Fery *et al.*, 2004).

For years, seed of photoperiod-sensitive accessions in the U.S. Department of Agriculture (USDA) cowpea germplasm collection has been regenerated at the USDA-ARS facilities in Isabela, Puerto Rico (Gillaspie *et al.*, 1999). Genetic diversity for tolerance to viral diseases has been observed by the

authors during these regeneration cycles. In this study, tolerance is defined 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 to determine 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 growers and/or breeders in cowpea improvement programmes.

MATERIALS AND METHODS

This study was conducted at the USDA-ARS Research Farm at Isabela, Puerto Rico. The Coto soil is a well-drained Oxisol (clayey, kaolinitic, isohyperthermic, Typic Hapludox). Relevant soil chemical properties in the evaluation plots during each year are presented in Table 1. The 25-year mean annual rainfall is 1649 mm, and Class A pan evaporation is 1672 mm. Mean monthly maximum and minimum temperatures are 29.8 and 19.9°C.

Field experiments were established on 1 February 2007 and 18 September 2008 using plant introductions (PI's) 180014, 441917, 441919, and 612607 and the commercial cultivar Coronet. PI's 441919 and 612607 were included in the study as virus-resistant controls for CMV and BICMV (Gillaspie, 2006). Cultivar Coronet was used as a susceptible control to both viruses (Gillaspie, 2002). PI 180014 was included as a high seed protein control

TABLE 1. Pre study soil characteristics at test plots in Isabela, PR, measured to a depth of 20 cm

Soil characteristics	Year	
	2007	2008
pH in water	5.8	5.8
Ammonium nitrogen (mg kg ⁻¹)	9.5	9.5
Nitrate nitrogen (mg kg ⁻¹)	19	19
Organic C (%)	0.94	1.15
P (mg kg ⁻¹)	53	52
K (cmol kg ⁻¹)	0.87	0.71
Ca (cmol kg ⁻¹)	1.89	2.03
Mg (cmol kg ⁻¹)	0.72	0.68

(Goenaga *et al.*, 2008). Phenotypic descriptors and origin of genotype are shown in Table 2.

The experiments were planted in a randomised complete block design with three replications. Because of the prostrate growth habit of some genotypes, experimental plots consisted of two 3-m rows spaced 3.6 m apart. Seedlings were thinned to an in-row spacing of 15 cm. Each experimental plot was surrounded by rows of the virus-susceptible cultivar Coronet which served as the source of the viruses' inoculum (i.e., spreader plants). On 27 February 2007 and 14 October 2008, all spreader-row plants were mechanically inoculated with leaf tissue extracts obtained from symptomatic plants of the cultivar Coronet. This tissue was previously determined to be infected with BICMV and CMV by the direct antigen-coated enzyme-linked immunosorbent assay (DAC-ELISA) as described by Gillaspie *et al.* (1995).

Inoculum was prepared by macerating excised trifoliolate leaves of the cultivar Coronet in 1% potassium phosphate buffer, pH 7.2 (1 g fresh tissue with 10 mL buffer). Spreader plants were then inoculated by dusting 320-grit Carborundum onto two trifoliolate leaves and applying the inoculum with a cotton swab. Inoculation of spreader plants was repeated 2 weeks after the first inoculation to reduce the possibility of plants escaping infection.

At about 65 days after planting, leaf samples from spreader plants were taken from eight random plants per row and sent by overnight mail to the Plant Genetics Resources Conservation Unit, Griffin, Georgia, USA, for virus testing by DAC-ELISA. This date was close to the midbloom period. In addition to BICMV and CMV, testing for the bromovirus *Cowpea chlorotic mottle virus* (CCMV), which is commonly found in Isabela, was also conducted. Experimental plants were not inoculated because of the possibility of virus resistance being the result of resistance to insects that transmit the viruses (e.g. aphids, bean leaf beetle). Fertilisation was provided 1 month after planting using a 16N-1.7P-3.3 K commercial mixture at a rate of 132 kg ha⁻¹. Plants were drip irrigated as necessary. Insect control was carried out with Malathion 57 EC (Loveland Products Inc., Greeley, CO) at recommended rates

TABLE 2. Phenotypic descriptors^a of five cowpea genotypes grown in Isabela, Puerto Rico

Genotype	Flower colour	Leaf shape	Plant habit	Dry pod colour	Pod placement	Pod Position	Pods per peduncle	Seed coat colour	Seed pattern colour	Seed pattern	Seed shape	Seed size	Seed coat texture	Origin
PI 441917	L	H	P	S	BF	P	2	T	GY	SP	S	L	S	Brazil
PI 441919	L	SH	P	S	BF	I	2	T	T	S	C	V	S	Brazil
PI 180014	L	SH	P	S	FL	P	3	B	B	S	S	M	M	India
PI 612607	W	SH	P	S	FL	P	2	W	B	E	N	L	R	Brazil
Coronet	W	H	SP	P	FL	P	2	W	P	E	N	M	M	United States

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

only during the pod filling period to prevent damage by pod worms.

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. Plant height was measured at midbloom with a ruler at midrow. Pods were harvested by hand as they matured, dried at 38 °C, 25% relative humidity, and shelled. 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 average 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 showed statistically significant ($P < 0.05$) genotype and year effects on most parameters measured in the study (Table 3). The genotype \times year interaction was not significant ($P > 0.05$) for grain

yield and other parameters; therefore these data were averaged and analysed over years.

ELISA results showed that all plots of the virus susceptible control 'Coronet' were infected with CMV, CCMV and BICMV in 2007. In 2008, all 'Coronet' plots were infected with BICMV and 2 out of 3 plots were infected with CMV; infection with CCMV in 2008 was minimal (Table 4). ELISA results for 'Coronet' spreader plants conducted on 7 March 2007 and 20 October 2008 showed the presence of the three viruses (data not shown). This suggests that virus infection of experimental plants occurred via insect transmission. Incidence of BICMV in all genotypes was higher in 2008 than in 2007.

ELISA readings for leaf samples of genotype 441917 demonstrated no CMV and BICMV infection in 2007, no CMV or CCMV infection in 2008 and, relative low absorbance values for BICMV in 2008 (Table 4). This genotype had the highest grain yield among genotypes, except for the virus resistant control PI 612607, which was not significantly different (Table 3). As compared to genotypes 180014, 441919 and cultivar Coronet, grain yield of PI 441917 was 57, 205 and 136% higher, respectively. These results confirm previous studies which showed that PI 441917 was an excellent yielder even when plants were infected with BICMV or CMV (Goenaga *et al.*, 2008).

TABLE 3. Yield components and other parameters of five cowpea genotypes in field experiments conducted at Isabela, Puerto Rico

Genotype	Grain yield (kg ha ⁻¹)	Seed protein (%)	100-seed weight (g)	Midbloom (days)	Maturity (days)	Plant height (cm)	Pod length (cm)
PI 441917	3682.8	22.8	20.2	48.5	66.3	48.0	23.7
PI 612607	2774.4	25.3	18.9	60.5	79.0	44.2	14.6
PI 180014	2348.4	26.1	8.5	48.8	63.7	49.8	14.2
PI 441919	1208.8	24.7	19.7	58.5	74.0	44.5	20.7
Coronet	1556.6	24.2	14.3	42.7	59.5	53.5	17.5
Average	2314.2	24.6	16.3	51.8	68.5	48.0	18.1
LSD (0.05) ^a	1095.2	1.4	1.6	2.8	1.8	3.7	1.1
Genotype (G)	**	**	***	***	***	***	***
Year (Y)	*	**	NS	*	**	*	NS
G \times Y	NS	NS	*	***	***	NS	NS

^aLeast significant difference at $P = 0.05$

NS, ***, **, * Nonsignificant or significant at P d⁰ 0.001, 0.01, or 0.05, respectively

TABLE 4. Absorbance readings for *Cucumber mosaic virus* (CMV), *Cowpea chlorotic mottle virus* (CCMV), and *Blackeye cowpea mosaic virus* (BICMV) in cowpea genotypes determined by direct antigen coating-enzyme linked immunosorbent assay in 2007 and 2008 from samples collected at approximately mid-bloom

Genotype	Replication	Year					
		2007			2008		
		CMV	CCMV	BICMV	CMV	CCMV	BICMV
PI 441917	1	0 ^b	0.2590	0	0	0	0.3465
PI 612607 ^a	0	0	0	0	0	0.5260	
PI 180014		0	0.3135	0.6735	0	0	1.2350
PI 441919		0	0	0	0	0	0.8825
Coronet		0.2040	1.5125	0.1560	1.1365	0	0.9640
PI 441917	2	0	0	0	0	0	0.3955
PI 612607 ^a		0	0	0	0	0	0.3695
PI 180014		0	0	0.4450	0	0	1.4435
PI 441919		0	0	0	0	0	0.6185
Coronet		0.3455	1.7890	0.1730	0.2880	0	0.5990
PI 441917	3	0	0	0	0	0	0.3485
PI 612607 ^a		0	0.1075	0	0	0	0.5185
PI 180014		0.1450	0.3230	0.5915	0	0	1.3740
PI 441919		0	0	0	0	0	1.0740
Coronet		0.1355	1.2765	0.2020	0	0.1100	0.7510

^a Cowpea genotype reported by Gillaspie (2002) to have CMV and BICMV resistance. ^b Zero absorbance denotes absence of virus

All plots of PI 61207 were infected with BICMV (Table 4) which contrasts the results obtained by Gillaspie (2002) describing this genotype as resistant to this virus. PI 180014 had high absorbance readings for CCMV and BICMV in 2007 and for BICMV in 2008, but grain yield was not significantly different than that for PI612607 (Tables 3 and 4).

PI 441919 was only affected by BICMV in 2008 but grain yield was low and not significantly different than that for the susceptible control 'Coronet'. The grain yield obtained by PI 441917 in this study (Table 3) was more than twice the mean yield (1424 kg ha⁻¹) of 25 improved, early-maturing varieties grown with good management in West Africa (Singh *et al.*, 2003).

Genotype 441917 had a significantly lower seed protein concentration than other genotypes (Table 3). As compared to the high-seed protein control, PI 180014, PI 441917 had a 12.6% lower seed protein concentration. An inverse relationship between seed yield and seed protein

concentration was established for other legume crops (Cober and Voldeng, 2000; de Mello Filho *et al.*, 2004).

Genotype 441917 had the highest 100-seed weight, although it did not differ significantly from PI's 441919 and 612607 (Table 3). Midbloom mean for all genotypes was 51.8 d (Table 3). However, cultivar Coronet took significantly shorter time to attain midbloom and maturity than other genotypes. Cultivar Coronet and PI 180014 were significantly taller genotypes (51.6 cm), but there was no significant difference in plant height between PI's 180014 and 441917. Among the genotypes used in the study, PI 441917 produced significantly longer pods, averaging 23.7 cm in length (Table 3).

In conclusion, PI 441917 outperforms all other genotypes for grain yield when it is grown under virus pressure, particularly BICMV. This PI should be useful to growers and to breeding programmes seeking to control yield losses caused by viruses, particularly BICMV and CMV.

Seed of PI 441917 is deposited in the U.S. National Plant Germplasm System where it is available for research purposes, including development and commercialisation of new varieties/cultivars. A limited quantity of seed of PI 441917 may be obtained by writing to orders@ars-grin.gov or to the principal author.

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