UARK-M2: A Line of Cowpea Cultivar Coronet with Blackeye Cowpea Mosaic Virus Resistance

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Cowpea [*Vigna unguiculata* (L.) Walp.] production in the southern United States is threatened by cowpea stunt disease. Characteristic symptoms of this disease include shortened internodes, collapsed and necrotic stems and petioles, leaf blistering, and little or no flowering. The causal agents of cowpea stunt are two unrelated plant viruses, blackeye cowpea mosaic potyvirus (BICMV) and cucumber mosaic cucumovirus (CMV), that interact synergistically in dually infected plants (Pio-Ribeiro et al., 1978). Both viruses are seed- and aphid-borne and can be transmitted separately or concomitantly.

The most effective means of controlling cowpea stunt is through plant resistance. While a number of BICMV-resistant cultivars exist (Kuhn et al., 1984; Miller and Scheuring, 1994; Pio-Ribeiro and Kuhn, 1980), no source of resistance to the cowpea stunt-associated isolates of CMV has been identified. Unfortunately, the most widely grown pinkeye, purple hull-type cultivar, 'Coronet', is highly susceptible to both of these viruses.

Three BICMV-resistant cultivars, 'Pinkeye Purple Hull-BVR', 'White Acre-BVR', and 'Corona', were released by the Univ. of Georgia in 1984 (Kuhn et al., 1984). These cultivars were developed by selecting BICMV-resistant plants from heterogenous populations of the three commercial cultivars 'Pinkeye Purple Hull', 'White Acre', and 'Coronet'. While the BICMV-resistant selections of 'Pinkeye Purple Hull' and 'White Acre' were indistinguishable from the susceptible parents, 'Corona' pods and seeds were significantly larger than those of 'Coronet' (Kuhn et al., 1984). Furthermore, BICMV-resistant cul-

tivars have not replaced 'Coronet' due to differences in seed characteristics, yield potentials, and other horticultural traits. For example, 'Pinkeye Purple Hull-BVR', like 'Pinkeye Purple Hull', is more susceptible than 'Coronet' to bacterial blight (*Xanthomonas campestris* pv. *vignicola*).

The purpose of this study was to use an approach similar to that employed by Kuhn et al. (1984) to select healthy cowpea plants from commercial fields of 'Coronet' that exhibited a near 100% incidence of cowpea stunt disease. The goal of this selection process was to obtain virus-resistant plants that were otherwise indistinguishable from 'Coronet' with respect to plant type, yield potential, and seed characteristics.

Origin

UARK-M2 originated from single plant selections of symptomless 'Coronet' plants taken from commercial fields where a high incidence of cowpea stunt was observed. Several hundred acres of 'Coronet' cowpea in the Missouri bootheel region were scouted during Summer 1995. Fields were observed at ≈ 6 weeks after planting and determined to be nearly 100% infected by cowpea stunt viruses. Fields were again scouted when pods were nearly mature. At that time, six asymptomatic plants were identified and subsequently harvested. These six selections were increased by seed propagation in a greenhouse by self-pollination through two generations. Progeny from the six selections were kept separate and continually evaluated for desirable horticultural characteristics, the presence of seed-borne virus, and disease response to CMV and BICMV. Progeny from one selection, designated UARK-M2, were found to be highly resistant to BICMV.

Description

No seed-borne BICMV was detected in any of the six selections based on visual observations of seedlings grown from collected seed, and by enzyme-linked immunosorbent assay (ELISA) performed on seedlings according to previously described methods (Anderson et al., 1996). Of the six selections, however, only one showed no inci-

dence of seed-borne CMV, and plants grown from all six selections were readily infected following mechanical inoculation with a cowpea stunt-derived isolate of CMV. A small number of progeny (6 to 12) from each of the six original plant selections was inoculated with a cowpea stunt-derived isolate of BICMV. No symptoms developed on UARK-M2 plants and no virus could be detected by ELISA. In addition, the UARK-M2 plants were very similar in appearance to 'Coronet' plants grown in the greenhouse. Seeds were collected from these plants to conduct a more detailed analysis of BICMV resistance. A crude sap extract from BICMV-infected 'Coronet' plants, prepared by grinding leaf tissue in 0.05 M phosphate buffer (pH 7.2) at an approximate ratio of 1:10 (mass/vol), was used to inoculate four to twelve 8-day-old plants in each of six separate greenhouse experiments. Plants were evaluated by observing symptom development for 14 to 21 days after inoculation. UARK-M2 plants were compared with the susceptible cultivar 'Coronet' and with the resistant cultivars Corona and Pinkeye Purple Hull-BVR. At 15 days postinoculation, the second set of trifoliate leaves from all plants was harvested and evaluated by ELISA for the presence of BICMV. A portion of the sap extracted for ELISA was diluted (1:20 v/v) in 0.05 M phosphate buffer (pH 7.2) and used to inoculate healthy 'Coronet' and *Chenopodium quinoa* (L.) plants for infectivity tests, with the presence of infectious virus being determined through the expression of systemic symptoms and local lesions, respectively.

In all tests, BICMV-inoculated 'Coronet' expressed strong systemic symptoms and supported relatively high levels of ELISA-detectable BICMV, while 'Corona', 'Pinkeye Purple Hull-BVR', and UARK-M2 plants expressed no symptoms and harbored no ELISA-detectable virus (data not shown). Furthermore, no symptoms developed on 'Coronet' and *C. quinoa* plants that were inoculated with sap extracts from the BICMV-resistant cultivars and UARK-M2, but systemic symptoms and local lesions were observed on 'Coronet' and *C. quinoa*, respectively, following back inoculations from BICMV-infected 'Coronet' plants. Finally, while 'Coronet' plants supported BICMV infections after being fed upon by viruliferous aphids, the 'Corona', 'Pinkeye Purple Hull-BVR', and UARK-M2 plants remained healthy based on visual observations and ELISA (data not shown).

A small percentage of BICMV-inoculated UARK-M2 plants were found to contain CMV when ELISA and infectivity tests were performed according to previously described procedures (Anderson et al., 1996). Because the inoculum source did not contain CMV, it was concluded that these UARK-M2 plants had grown from seeds harboring CMV. The fact that cowpea stunt disease did not develop on CMV-infected plants following inoculation with BICMV provided further evidence of an extremely high level of resistance to BICMV that resulted in excellent protection from cowpea stunt disease.

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Table 1. Mean pod length, seed per pod, yield, and seed mass of UARK-M2 and three commercial cultivars (controls) from a 1996 field test.

Cowpea genotype	Pod length (cm) ^z	Seed per pod ^y	Yield (kg·ha ⁻¹) ^x	Mass per 100 seed (g) ^w
UARK-M2	16.6	10 a	1576 a	18.9 a
Coronet	15.9	11 a	1728 a	18.3 a
PPH-BVR ^v	16.8	11 a	1376 a	18.9 a
Corona	16.2	7 b	763 b	28.3 b

^zMeans for 74 dry pods randomly sampled from total harvests. F test for differences among genotypes was nonsignificant at $P > 0.05$.

^yMeans for the same 74 pods used to determine mean pod length.

^xExtrapolated from 2 replicate plots.

^wMeans for two groups of 100 dry seed.

^vPPH-BVR = 'Pinkeye Purple Hull-BVR'.

Mean separation by LSD ($P > 0.05$).

Fig. 1. Mature, dry seed of field-grown 'Coronet' and UARK-M2 cowpea.

Performance

A replicated, small plot field test was conducted to compare plant type and yields of UARK-M2 with 'Coronet', 'Pinkeye Purple Hull-BVR', and 'Corona'. A randomized complete-block design was used with two replications, where each replication was a single 3-m (10 ft) section of a 6-m (20 ft) row. The test was conducted from June through Aug. 1996 at the Univ. of Arkansas, Agricultural Experiment Station Farm, Fayetteville. Data were analyzed by an analysis of variance. UARK-M2 plants were indistinguishable from 'Coronet' and matured at about the same time (78 days

after planting). Average pod length did not differ significantly among genotypes, but 'Corona' had fewer seed per pod compared with the other genotypes (Table 1). Under normal field conditions, 'Pinkeye Purple Hull-BVR' generally yields less than 'Coronet'. In this study, the yields of UARK-M2, 'Coronet', and 'Pinkeye Purple Hull-BVR' did not significantly differ from each other, but were significantly higher than that of 'Corona' (Table 1). In addition, two samples of 100 dry seed from each genotype were chosen at random and weighed. There was no significant difference in the average masses of UARK-M2, 'Coronet', and 'Pinkeye Purple Hull-BVR'

seeds, but 'Corona' seed were significantly larger than those of the other three genotypes (Table 1).

Taken together, the data presented here indicate that, except for striking differences in their response to BICMV, there were no significant horticultural differences between UARK-M2 and 'Coronet' plants. Seed of UARK-M2 and 'Coronet' were essentially indistinguishable with respect to size, shape, and color (Fig. 1).

Because of its similarity to 'Coronet' and high level of resistance to BICMV, UARK-M2 should be a valuable germplasm line for cowpea breeding programs and might be used directly as a BICMV-resistant line of 'Coronet'.

Availability

A limited quantity of UARK-M2 seed for breeding purposes can be obtained through written request to A.S.K or E.J.A. at the Dept. of Plant Pathology, Univ. of Arkansas, 217 Plant Science Building, Fayetteville, AR 72701. This breeding line is released without restriction.

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