

Inheritance of resistance to blackeye cowpea mosaic virus in *Vigna unguiculata*

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ABSTRACT: Crosses between the resistant cowpea line TVU 2480 obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and the susceptible domestic cultivar Early Ramshorn were used to determine the inheritance of resistance to blackeye cowpea mosaic virus (B1CMV). Evaluation of F₁, F₂, and reciprocal backcross populations by symptomatology and enzyme-linked immunosorbent assay (ELISA) clearly indicated that a single recessive gene controls the high level of resistance. The symbol *bcm* (blackeye cowpea mosaic) is assigned to this factor. IITA cowpea lines TVU 2740, TVU 3273, TVU 2657, and TVU 2845 seem to possess this same gene.

IN AN EARLIER STUDY, Pio-Ribeiro et al.⁷ failed to identify the inheritance of resistance to blackeye cowpea mosaic virus (B1CMV) in crosses of the resistant cowpea cultivar Iron with the susceptible California Blackeye, due to the absence of resistant plants in the F₂ generation. In a recent study involving the comparison of isolates of this virus with those of cowpea aphid-borne mosaic virus (CAMV), better sources of resistance to B1CMV were identified⁸. This paper presents evidence that in *Vigna unguiculata* (L.) Walp., line TVU 2480, resistance to B1CMV is governed by a single recessive factor.

Materials and Methods

Genetic populations for this study were derived from crosses between line TVU 2480 [International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria] which had been shown to be resistant to isolates of B1CMV from Florida, New York, Kenya, and Nigeria⁸, and the susceptible cultivar Early Ramshorn. Uniform germination of the parental, F₁, F₂, and reciprocal backcross seed was insured by scarifying and pregerminating them on moist blotter paper in petri dishes. Seedlings were individually transplanted into pots and maintained subsequently in an insect-free greenhouse kept at 24-27°C. All plants were mechanically inoculated 7 and 15 days after transplanting with an inoculum prepared by triturating leaves of Early Ramshorn cowpea systemically infected with the New York isolate of B1CMV⁹, using 0.05 M phosphate

buffer (K⁺) at pH 7.5. The double inoculation was to ensure infection in all the susceptible genotypes. Plants were kept under observation for a 28-day period, after which they were checked for the presence of the virus using enzyme-linked immunosorbent assay (ELISA) as described by Clark and Adams², and modified by Taiwo⁶.

Results

Plants of the resistant parent, TVU 2480, did not become infected with B1CMV and the virus was not recovered from inoculated primary leaves and uninoculated trifoliolates. Plants of Early Ramshorn cowpea developed veinal chlorosis, green vein banding, and some browning of the leaves.

As shown in Table I, all the F₁ plants developed systemic symptoms, and those of F₂ populations segregated in a ratio of nearly three susceptible to one resistant. The cross of F₁ to the resistant parent resulted in progeny segregating in a ratio of one resistant to one susceptible, while the F₁ crossed to the susceptible parent gave progeny that were susceptible. Resistant progeny were proved to possess the same high level of resistance of the parent TVU 2480. With a few exceptions, the results obtained by visual observation were matched with the ELISA results. The data indicate that resistance to B1CMV in TVU 2480 is conferred by a single recessive gene, to which the symbol *bcm* (blackeye cowpea mosaic) is assigned.

Discussion

The failure of Pio-Ribeiro et al.⁷ to detect any resistant plants in their F₂ population could be due to the different reactions of the cultivar Iron to isolates of B1CMV. Kuhn et al.^{3,4} reported that Iron was susceptible to an isolate of the virus from Georgia, which re-

sembled that described by Anderson¹ in Florida. Similarly, Pio-Ribeiro and Kuhn⁶ found that Iron succumbed to infection by a B1CMV isolate from White Acre cowpea seed produced in Georgia in 1959 and another isolate from South Carolina. Furthermore, the same researchers noted that a small percentage of Iron plants were susceptible to the isolate of B1CMV used for inheritance studies⁷. Consequently, it appears that Iron is not a homogeneous population and that its resistance may be strain specific.

The high level of resistance in cowpea line TVU 2480 represents valuable germplasm material that can be easily used in breeding programs for the development of new viral resistant cultivars of *V. unguiculata*. It is noteworthy to mention that this resistance also is functional against isolates of B1CMV from Africa and probably Asia⁸.

In addition to TVU 2480, resistance to the same isolates of B1CMV was found in four other IITA lines (TVU 2740, TVU 3273, TVU 2657, and TVU 2845). In F₁ plants of these lines, resistance was also recessive, suggesting that the *bcm* gene is involved. Unfortunately, it was not possible to analyze the F₂ generations since plants failed to flower under our conditions due to a long photoperiod (16-hour daylight).

Recent studies by Taiwo⁸ confirmed that B1CMV and CAMV are two distinct viral entities⁵, for which no common sources of resistance are presently available in *V. unguiculata*. Since it has been proved that in addition to the United States, B1CMV also occurs in Kenya, Nigeria, and Japan⁸, it is conceivable that this virus and CAMV already coexist wherever the cowpea crop is cultivated. Both viruses are seed-borne and are readily transmitted by several species of aphids. The free exchange of seed has probably greatly facilitated their introduction into areas where one or both may have been previously

Table I. Data on inheritance of resistance to blackeye cowpea mosaic virus in cowpea

Progeny	No. plants		Expected ratio	Goodness-of-fit (P)
	resistant	susceptible		
Early Ramshorn (E.R.)	0	100		
IITA* TVU 2480	60	0		
2480 × E.R. F ₁	0	20		
E.R. × 2480 F ₁	0	22		
(1) 2480 × E.R. F ₂	23	96	1:3†	0.16
(2) 2480 × E.R. F ₂	25	90	1:3†	0.44
(3) 2480 × E.R. F ₂	26	92	1:3	0.47
(1) E.R. × 2480 F ₂	17	51	1:3‡	1.00
(2) E.R. × 2480 F ₂	18	80	1:3	0.14
(3) E.R. × 2480 F ₂	22	73	1:3	0.58
(2480 × E.R.) F ₁ × 2480 BC ₁	19	24	1:1	0.56
(2480 × E.R.) F ₁ × E.R. BC ₁	0	13	0:1	1.00

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† 18 seed lost due to lack of germination or root rot

‡ 19 seed lost due to lack of germination or root rot

§ 5 seed lost due to lack of germination or root rot

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absent. Thus, for an effective genetic control of both B1CMV and CAMV, resistance to one must be augmented by the resistance to the other. Sources of resistance to CAMV in *V. unguiculata* also are available⁸ and studies will be conducted to determine the mode of inheritance.

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Heritability of water-loss rate in *Drosophila melanogaster*

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ABSTRACT: Twenty-eight isofemale lines of *Drosophila melanogaster* newly collected from Brownsville, Texas were used to study the genetic component of water loss rates. Two standard laboratory stocks were used to determine basic water balance features of *D. melanogaster*. The laboratory stocks show greater loss rates at low humidities than at high ones, but neither age nor strain differences are evident. Crosses within and among the isofemale lines, both from standard cultures and from highly inbred lines, show that there is a genetic component of water loss rate, which is greater in males than in females. Regressions of offspring on midline parent values gave heritability estimates of a water loss rate of 0.33 for males, and 0.17 for females.

ALTHOUGH the ability to remain-in-water balance is important to adaptation and survival in *Drosophila* species^{1,2,5,9}, little is known about the genetics of its control. Presumably, this trait is under polygenic control and is subjected to natural selection; however, this hypothesis has not been thoroughly tested. Parsons⁵ estimated the heritabilities of three characters related to water balance—weight, dry weight, and survival—to be 0.402, 0.411, and 0.600, respectively. Steiner⁹ determined that the heritability for desiccation resistance in *D. mimica*, a Hawaiian species, is 0.54.

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Water-loss rate (percent body water lost each hour) may be used as a measure of adaptation to desiccating environments because selection can act directly on such a character and because it reflects specific morphological and physiological features, such as cuticle thickness and waxiness and the ability to control spiracular water loss. Water-loss rate is related to survival time at low humidities and may play a role in population structure^{3,4}. Parsons⁵ demonstrated that the spatial distribution of flies in the field depends largely on humidity and water availability. Because water balance may influence population structure and, therefore, affect breeding behavior, its role in evolution might be important. This study was designed to determine if water-loss rate is under genetic control and, if so, to estimate the narrow sense heritability of water-loss rate in *Drosophila melanogaster*.

Materials and Methods

Water-loss rates were determined as described in Eckstrand and Richardson², and all experiments were carried out at 20°C. Virgin males and females were collected and aged in shell vials papered with damp tissues for 12 hours. Each fly was then individually weighed, housed in a small glass chamber approximately (0.5 ml), and placed at the test humidity⁸. After 6 hours, it was removed and reweighed. Water-loss rate was calculated as the average percentage of initial body weight lost each hour.

The *D. melanogaster* lines used in these studies were as follows:

1. *cn bw*: a standard chromosome 2 line marked with two recessive eye-color mutants, *cn* (cinnabar eye color, 2R-57.5) and *bw* (brown eye color, 2R-104.5).
2. *Tokyo*: a wild-type line originally isolated from a Tokyo, Japan population more than 20 years ago.
3. *Brownsville*: 28 isofemale lines collected at Brownsville, Texas in July 1979.

Four sets of experiments were carried out to determine if water-loss rates are under genetic control.

1. *Within lines*. Two males and two females from each of the 28 *Brownsville* lines were tested and pair mated. Five male and five female offspring from each of the two pair matings were also tested.
2. *Among lines*. Four males and females from each of the 28 *Brownsville* lines were tested. Then, pair matings were made among individuals from different *Brownsville* lines. Reciprocal matings were also made. Five male and five female offspring from each cross were tested.
3. *Within inbred lines*. Gravid females were selected from each of the 28 *Brownsville* lines and individually placed in culture vials. Twenty inbred lines were subsequently maintained by sib-sib mating each generation for seven generations prior to testing.
4. *Among inbred lines*. Individuals from 11 of the inbred lines were crossed among lines. Five male and five female offspring from each cross were tested for water loss.

Results

Between two days and two weeks of age, flies showed no consistent change in water-loss rate for either the *Tokyo* or the *cn bw* stocks (Table I). The *cn bw* stock had slightly lower loss rates than the *Tokyo* stock at high *a_v*'s and some individuals apparently sorbed water at 0.99 *a_v*. The data were characterized by considerable variation in loss rate between individuals at all *a_v*'s tested.

Table II shows the results of the analyses of variance for the progeny from each of the four experimental crosses involving the *Brownsville* strains. The progeny from within-lines crosses did not differ with respect to either parental strain or sex. Neither was there any significant interaction between strain and sex. However, when the parents came from different lines (among-lines crosses) there were significant differences between progeny with respect to strain. Neither the component of variation attributable to sex nor the interaction term were significant, however.

Seven generations of inbreeding led to a