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INHERITANCE OF RESISTANCE IN COWPEA [*VIGNA UNGUICULATA* (L.) WALP.] TO BLACKEYE COWPEA MOSAIC POTYVIRUS

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Abstact

In order to determine the mode of inheritance of resistance to blackeye cowpea mosaic virus in 6 cowpea lines, direct, reciprocal and back crosses were attempted between resistant and susceptible parents. The data from F_1 populations suggested the dominant nature of susceptibility. The number of resistant and susceptible plants in F_2 progenies of each cross segregated in a ratio of 1 resistant : 3 susceptible. The observed ratios were compared with the expected monogenic recessive model for goodness of fit using chi-square test. Based on the results obtained from F_1, F_2 and back crosses, it is concluded that the resistance in the 6 cowpea lines viz., IT86F-2089-5, IT86D-880, IT90K-76, IT86D-1010, IT86F-2062-5 and BP1CP3 is conditioned by a single homozygous recessive gene. The symbol for this gene is proposed as "bcm" (blackeye cowpea mosaic).

Introduction

Cowpea {*Vigna unguiculata* (L.) Walp.} is an important legume crop grown in tropical and sub-tropical countries of the world. The diseases caused by viruses are among the major factors which contribute for low yields of cowpea. Virus diseases cause serious losses of yield and quality in cowpea in many cowpea growing countries. Worldwide, more than 20 viruses have been identified which infect cowpea under field or experimental conditions (Thottappilly & Rossel, 1985; Mali & Thottappilly, 1986). Numerous viruses are infectious to cowpea and are considered potential natural threat to cowpea production (Kuhn, 1990). In Pakistan 5 seed transmitted viruses are reported in cowpea (Bashir & Hampton, 1993). Among the seed transmitted viruses, blackeye cowpea mosaic (BlCMV) is more serious than others and can cause significant economic losses when occurs in epidemic proportions.

Breeding for resistance has become an increasingly common practice on the development of methods for the control of viral diseases in economically important crop plants. The use of resistant genes provide an effective and economical solution of such viral diseases. Resistant sources are available against BlCMV (Kuhn, 1990; Bashir & Hampton, 1995, Bashir *et al.*, 1995).

Keeping in view the importance of resistant genes and their use in effective breeding programme for the development of resistant cowpea cultivars, this study was conducted with the objective to determine the genetic basis of resistance in 6 cowpea genotypes viz., IT86F-2089-5, IT86D-880, IT90K-76, IT86D-1010, IT86F-2062-5, and BP1CP3, which were found highly resistant to BlCMV (Bashir *et al.*, 1995)

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Materials and Methods

Selection of parents: Six cowpea genotypes resistant to BICMV Viz., IT86F-2089-5, IT86D-880, IT90K-76, IT86D-1010, IT86F-2062-5, and BP1CP3, besides 'Pusa Phalguni' susceptible to BICMV were selected for crossing purpose. The seeds of the first 5 cowpea lines were obtained from International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The seeds of BP1CP3 were obtained from International Rice Research Institute (IRRI), Philippine. The seed of 'Pusa Phalguni' was already available with us.

Maintenance of virus culture: An isolate of BICMV which was originally obtained from infected cowpea seed (Bashir *et al.*, 1995) was used in this study. The isolate was maintained by infrequent mechanical inoculation on susceptible cowpea plants in insect-free glasshouse. Additionally, the virus isolate was also maintained in infected cowpea seeds stored at 4°C.

Planting procedure: All the experiments conducted during this study were carried out in an insect-free greenhouse which was sprayed weekly with insecticide Monitor 600 EC to control aphids. Fifty seeds of each parent (6 resistant and 1 susceptible) were planted in (40 cm diameter earthen pots, The pots were @5 seeds/pot), filled with sterilized soil mixture of silt, sand and farm yard manure in a ratio of 2:1:1, respectively. The pots were kept in the greenhouse and were maintained at 28-30°C and light (14 hr. photoperiod). The plants were regularly watered and properly fertilized when required.

Crossing procedure: The flowers on the resistant and the susceptible parents were emasculated between 1700 and 1900 hr using forceps and properly tagged. Next day early in the morning the flowers were pollinated with pollen grains collected from their respective parents. Crossing procedure was the same as adopted by Quattara & Chambliss (1991). Reciprocal and back crosses were also attempted. The hybrid seeds were properly collected at maturity and maintained crosswise.

Screening of parents against virus: Fifty plants of each parent were tested by mechanical inoculation under insect-free glass conditions. When the seedlings were 2 weeks old, virus inoculum prepared in 0.01 M phosphate buffer with pH 7.0 was applied on primary leaves dusted with Carborundum powder (600 mesh). The plants were washed with water after inoculation. The same plants were reinoculated 2 weeks later to avoid an escape. Disease symptoms were recorded at 2 weeks interval. The symptomless plants were tested by enzyme-linked immunosorbent assay (ELISA).

Screening of F_1 progeny against virus: The F_1 progeny of each cross was raised from hybrid seeds while growing in earthen pots. The plants were mechanically inoculated with virus BICMV inoculum according to the method described by Bashir *et al.*, (1995), when the seedlings of F_1 progenies were 8-10 days old and the primary leaves were fully expanded. Ten plants of each parent (resistant and susceptible) were also inoculated at the same time with the same inoculum. Non-inoculated plants of each parent served as negative control. Two weeks after first inoculation all the plants were reinoculated to avoid any escape. The inoculated plants were kept under observations in the greenhouse till maturity.

Susceptibility/resistance criteria: All the inoculated plants of F_1 progeny of each cross and control plants were observed at 15 days interval for disease appearance. The indi-

vidual plant of each treatment was scored for the presence of virus symptoms using 0-4 scale (Bashir *et al.*, 1995). The plants showing mild to severe systemic symptoms were considered as 'susceptible'. The symptomless plants of each treatment were assayed for virus infection using direct antigen coating-enzyme-linked immunosorbent assay (DAC-ELISA) according to the procedure as described by Hobbs *et al.*, (1987). The symptomless plants with no virus detection by ELISA were considered as 'resistant' to BlCMV.

Evaluation of F_2 progenies: The seeds obtained from each F_1 cross (direct, reciprocal and back crosses) were grown in earthen pots filled with sterilized soil. After one week of planting the primary leaves of the seedlings were inoculated with virus culture. Inoculum was prepared and applied as has been described before. After 3 weeks of first inoculation, the symptomless plants of each cross were reinoculated to ensure virus infection and to avoid any escape. Individual plant of each cross was scored for disease severity following 0-4 point scale at 15 days interval. After 5 weeks of first inoculation the leaf samples were taken from the symptomless plants and were tested by ELISA to detect virus and to separate resistant from susceptible plants.

Application of chi-square test: The procedure followed to compute chi-square values of the data obtained from F_2 segregation population of each cross was followed as described by Gomes & Gomes (1984).

Results

Screening of parents: The following 6 parents viz., IT86F-2989-5, IT 86D-880, IT90K-76, IT86D-1010, IT 86F-2062-5 and BP1CP3 were found highly resistant. All the plants of these parents were found symptomless and no virus was detected when tested by ELISA. In case of "Pusa Phalguni" all the plants were found susceptible to the virus.

Testing of F_1 progenies: In F_1 tests, all plants in the 6 crosses (direct and reciprocal) were found susceptible to BlCMV (Table 1). F_1 plants developed systemic infection with characteristic disease symptoms similar to susceptible parent indicating that resistance was inherited recessively. This condition was confirmed by the reaction of F_2 progenies, which segregated in the ratio 1 resistant : 3 susceptible. However, it was observed that the inoculated plants in reciprocal crosses exhibited more disease severity than the plants of indirect crosses. Variation in disease symptoms (mild to severe) was observed not only among the crosses but also within the same cross. Virus was recovered in high titer in plants showing mild systemic infection in some of the crosses when tested by ELISA.

Reciprocal F_1 populations from all crosses (Table 1) were also susceptible to BlCMV, with no maternal (cytoplasmic) effect. Expression of susceptibility to BlCMV in reciprocal F_1 populations was identical to F_1 from direct crosses. Based on disease symptoms recorded on individual plant and ELISA results it was found that all the plants of F_1 of each cross were susceptible to BlCMV, indicating that susceptibility was dominant over resistance suggesting the monogenic recessive model of inheritance.

Back cross populations: The F_1 obtained from each direct cross was also back-crossed with susceptible male parent 'Pusa Phalguni'. It was observed that seed setting in back crosses was poor as compared to other crosses attempted. However, a reasonable

number of seeds were obtained to have meaningful results for final conclusion. All the plants tested under each back cross were susceptible to the virus (Table 1). ELISA results also correlated with disease symptoms. The results of back crosses also supported a single recessive gene inheritance model.

Evaluation of F_2 population (direct crosses): The F_2 population from each direct cross segregated in the ratio 1 resistant: 3 susceptible. All the symptomless plants of each cross when tested by ELISA, virus was not recovered. Throughout the trials we observed variability in disease symptoms ranging from mild to severe infection not only among the crosses but also within the same cross. Virus titer was also different in plants of the same cross. On the average ELISA results were highly correlated with the visual symptom scoring. ELISA reading for known positive (1.823 - 2.748) and negative (0.021 - 0.034) samples indicated adequate separation of BlCMV-infected (susceptible) and symptomless non-infected (resistant) plants. Irrespective of the disease symptoms (mild to severe) and virus titer variability in plants of the same cross, this group of plants was considered as 'susceptible'.

Evaluation of F_2 populations (reciprocal crosses): Six reciprocal crosses were also attempted using susceptible parent as a female and resistant parent as a male. Cytoplasmic effect towards resistance was not observed. F_2 population of each cross in reciprocal crosses also segregated in a ratio of one resistant: 3 susceptible on the same pattern as was obtained in direct crosses.

The number of resistant and susceptible plants obtained in each cross of direct and reciprocal crosses were compared with those expected in monogenic recessive model for major gene and goodness of fit using chi-square test (Gomes & Gomes, 1987). Chi-square (x^2) values calculated on the basis of observed ratio (1 : 3) of each cross are given in Table 1. As the calculated Chi-square (x^2) value of each cross is less than the tabulated values at 5 % or 1 % level of probability, which indicated that the results are non-significant. These segregation (all observed ratios 1 resistant: 3 susceptible) fit the hypothesis that resistance in these 6 cowpea genotypes is dependent upon the homozygous condition of a single recessive gene pair.

Based on the results obtained from F_1, F_2 segregation populations, and back crosses, it is concluded that resistance in the following 6 cowpea genotypes viz., IT86F-2089-5, IT86D-880, IT90K-76, IT86D-1010, IT86F-2062-5 and BP1CP3 is conditioned by a single recessive gene for which we proposed the same symbol 'bcm' (blackeye cowpea mosaic) which has already been suggested by Walker & Chambliss (1981).

Discussion

Breeding for resistance is one of the most economical and effective means of controlling virus diseases of plants. Resistance resources to BlCMV have been identified by several workers (Bashir *et al.*, 1995; Mali *et al.*, 1988; Taiwo *et al.*, 1982). Recently 10 cowpea genotypes were identified as highly resistant to a local isolate of BlCMV in Pakistan (Bashir *et al.*, 1995) and 6 of them were included in this study to determine the genetic basis of resistance in these cowpea lines. Perhaps understanding the mode of inheritance of resistance to BlCMV in these cowpea lines will help to

Table 1. Segregation ratios and chi square analysis of cross and back cross populations of cowpea genotypes resistant and susceptible to blackeye cowpea mosaic virus.

Parent/cross	No. of plants tested	Susceptible	Resistant	Expected ratio	X^2 value	df	Goodness of fit (Probability)
Resistant parents							
IT 86F-2089-5 (V1)	50	0	50	-	-	-	-
IT 86D-880 (V2)	50	0	50	-	-	-	-
IT 90K-76 (V3)	50	0	50	-	-	-	-
IT 86D-1010 (V4)	50	0	50	-	-	-	-
IT 86F-2062-5 (V5)	50	0	50	-	-	-	-
BPICP3 (V6)	50	0	50	-	-	-	-
	40	0	40	-	-	-	-
Susceptible parent							
Pusa Phalguni (S)	50	50	0	-	-	-	-
F_1 (Direct cross)							
V1 X Pusa Phalguni	17	17	0	-	-	-	-
V2 X Pusa Phalguni	39	39	0	-	-	-	-
V3 X Pusa Phalgyni	76	76	0	-	-	-	-
V4 X Pusa Phalguni	12	12	0	-	-	-	-
V5 X Pusa Phalguni	38	38	0	-	-	-	-
V6 X Pusa Phalguni	67	67	0	-	-	-	-
F_1 (reciprocal crosses)							
Pusa Phalguni X V1	39	39	0	-	-	-	-
Pusa Phalguni X V2	73	73	0	-	-	-	-
Pusa Phalguni X V3	18	18	0	-	-	-	-
Pusa Phalguni X V4	24	24	0	-	-	-	-
Pusa Phalguni X V5	18	18	0	-	-	-	-
Pusa Phalguni X V6	45	45	0	-	-	-	-
F_2 (direct crosses)							
V1 X Pusa Phalguni	192	142	53	1:3	0.061	1	0.95-0.80
V2 X Pusa Phalguni	269	196	73	1:3	0.214	1	0.20-0.05
V3 X Pusa Phalguni	959	737	222	1:3	1.647	1	0.20-0.05
V4 X Pusa Phalguni	41	31	10	1:3	0.008	1	0.95-0.80
V5 X Pusa Phalguni	327	2522	75	1:3	0.636	1	0.50-0.30
V6 X Pusa Phalguni	610	453	157	1:3	0.140	1	0.80-0.70
F_2 (reciprocal crosses)							
Pusa Phalguni X V1	406	303	103	1:3	0.012	1	0.95-0.80
Pusa Phalguni X V2	651	485	166	1:3	0.061	1	0.95-0.80

Table 1 (Cont'd)

Parent/cross	No. of plants tested	Suscep-tible	Resis-tant	Expected ratio	X^2 value	df	Goodness of fit (Probab-lity)
Pusa Phalguni X V3	263	198	65	1:3	0.01	1	0.99-0.95
Pusa Phalguni X V4	303	727	76	1:3	0.001	1	0.99-0.05
Pusa Phalguni X V5	161	118	43	1:3	0.167	1	0.70-0.50
Pusa Phalguni X V6	452	348	134	1:3	1.869	1	0.20-0.05
Back crosses							
(V1XS) X Pusa Phalguni	10	10	0	-	-	-	-
(V2XS) X Pusa Phalguni	38	38	0	-	-	-	-
(V3XS) X Pusa Phalguni	20	20	0	-	-	-	-
(V4XS) X Pusa Phalguni	15	15	0	-	-	-	-
(V5XS) X Pusa Phalguni	28	28	0	-	-	-	-
(V6XS) X Pusa Phalguni	22	22	0	-	-	-	-

develop an effective future breeding programme to evolve resistant cowpea cultivars with desirable characters.

During screening of F_1, F_2 and back cross progenies to virus infection symptom differences were apparent not only among the progenies from different crosses but also within the progeny of the same cross. Therefore, we recorded disease symptoms on individual plant using 0-4 scale. For convenience, the moderately susceptible and highly susceptible segregants in the F_2 population were pooled within each cross against resistant segregants to work out the fitness to 1 resistant: 3 susceptible. However, we could not detect virus by ELISA in symptomless plants found within each cross, which were regarded as resistant. Similar observations of differential symptoms on peas have been recorded as responses to inoculation with pea mosaic virus and other related viruses, and have been attributed to plant age, environmental conditions and genotypes, but not due to genetic factors (Yen & Fry, 1956).

Plants of F_1, F_2 and back cross populations were classified as resistant or as susceptible according to visible disease symptoms and testing by ELISA. All the plants from F_1 and back cross were susceptible, indicating that resistance is recessive to susceptibility. Considering the over all F_2 segregation pattern (1 resistant: 3 susceptible) for reaction to BlCMV seems to follow the hypothesis of single recessive gene model.

Based on the results obtained from F_1, F_2 and back cross populations of this investigation, we concluded that a single recessive gene controls resistance to BlCMV in the cowpea lines IT86F-2089-5, IT86D-880, IT90K-76, IT86D-1010, IT86F-2062-5 and BP1CP3. The same mode of inheritance was implicated in the resistance to BlCMV in cowpea cultivar Worthmore (Walker & Chambliss 1981). Taiwo *et al.*, (1981) also reported a single recessive gene responsible for high level of resistance in cowpea lines

TVu-2740, TVu-3273, TVu-2657 and TVu-2845. In contrast to these results a single dominant gene for resistance to BlCMV in cowpea cultivar "White Acre-BVR" (Quattara & Chambliss, 1991), bean cultivar (*Phaseolus vulgaris*) "Black Turtle Soup" (Provvidenti *et al.*, 1983); and cowpea cultivar "Pinkeye Purple Hull BVR" (Strniste, 1987) has been reported.

No doubt there are more chances for the breakdown of resistance controlled by major genes (Vertical resistance) by evolution of new virulent virus strain with the passage of time than the resistance controlled by polygenes (Horizontal resistance), but it is more convenient to transfer vertical resistance than horizontal resistance to develop improved cultivars.

Thus high level of resistance to BlCMV in the 6 cowpea lines can be exploited easily in breeding programme designed to transfer BlCMV- resistance to desirable commercial cowpea cultivars and to aid in the control of cowpea stunt disease which is caused by mixed infection of BlCMV and cucumber mosaic virus (CMV) under field conditions (Kuhn, 1990). It is interesting to note that all the resistant lines which we used in this study are also resistant to a local isolate of cowpea aphid-borne mosaic potyvirus (M. Bashir *un-published*).

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