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INHERITANCE STUDIES OF BACTERIAL BLIGHT DISEASE RESISTANCE GENES IN COTTON (*GOSSYPIUM HIRSUTUM* L.)

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Abstract

Two bacterial blight resistant varieties/strains of cotton viz., C_2 (67) 577 and C_2 (69) 1455 both of each used as male and female parents were crossed with the susceptible variety DPL-7344-424, to study the inheritance response of resistance genes for bacterial blight. The cross C_2 (67) 577 x DPL-7340-424 (resistant x susceptible) indicated a single gene difference with complete dominance for resistance to the race-18 in the resistant parent C_2 (67) 577. Single gene inheritance of resistance to the race-18 was also indicated in C_2 (67) 577 when crossed as male with susceptible DPL-7340-424. Both seedling and adult plant growth stages, F_2 and back cross disease grade distributions clearly indicated a monogenic type of inheritance of resistance in resistant x susceptible and susceptible x resistant crosses. The monogenic inheritance of resistance indicates that pedigree breeding would be adequate for transferring the resistance in the susceptible genotypes. All the plants were resistant in F_1, F_2 and back cross progenies in cross between resistant x resistant showings that the gene for resistance is the same in both the parents. Seedling and adult disease grade in the present study were positively correlated as monogenic inheritance was observed in both seedling and in the adult stage of growth. These results support the concept of common basic mechanism controlling the resistance in the two different stages of plant growth and suggest that selection for resistance can be accomplished in either growth stage.

Introduction

Cotton (*Gossypium hirsutum* L.) is main export and cash crop of Pakistan and plays a vital role in the economy of the country. The efforts have always been made for the genetic improvement of cotton to produce more seed cotton yield, better fiber quality and resistance to diseases and insect pests. Cotton crop has suffered from many severe viral, fungal and bacterial diseases in addition to various insect pests, which cause heavy yield losses (Khan & Rashid, 1996). Bacterial blight, caused by *Xanthomonas campestris* pv *malvacearum* (E. F. Smith) Dowson, is one of the major cotton diseases in Pakistan (Hussain & Ali, 1995). The investigation made on the bacterial blight of cotton revealed that this disease was seed and trash borne under local conditions. Resistant cultivars offer the most economical means of controlling bacterial blight. To produce resistant cultivars, information on the number of resistance genes and their pattern of inheritance is important.

Resistance to the bacterial blight pathogen in upland cotton breeding lines and cultivars is attributed to the contribution of two or more major bacterial blight resistance genes and a modifier complex (Bird, 1982; El-Zik & Bird, 1970; Girardot *et al*., 1986; Innes, 1974). Brinkerhoff *et al*., (1978) reported that inheritance of resistance to bacterial blight in cotton was due to a single dominant gene. Sahu & Khush (1989) investigated the

mode of inheritance and allelic relationship between resistant genes for bacterial blight in seven Rice cultivars. They revealed that ADT-22, Mondba, Hegurmanah-2 and BR-116-3B-53 varieties have Xa_4 and Xa_{10} resistant genes, whereas Arairay, BR-11 and Chhatari have only the Xa_{10} gene. Wallace & El-Zik (1989) determined the inheritance for resistance to a mixture of USA races and the HV-1 isolate of bacterial blight (*Xanthomonas campestris* pv *malvacearum*) in cotton and examined the relationship between resistance at the cotyledon stage with that of true leaf stage. The single gene with complete dominance for resistance in cotton to the USA race mixture was observed.

Nineteen races of the pathogen are recognized so far (Knight, 1946). Genetic studies of disease resistance conducted by various researchers (Wallace & El-Zik, 1989; Bird, 1974) showed that it is not simply inherited but it is affected by many genes with modifiers complex in some of diseases. In some cases it is controlled by major dominant gene with one or more modifiers (Girardot *et al*., 1986). The objective of this study was to determine mode of inheritance of bacterial blight disease resistance genes in highly resistant cotton genotypes, and to determine the genetic relationship between these sources of resistance.

Materials and Methods

The studies were conducted in collaboration with Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad; National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad and Central Cotton Research Institute (CCRI), Multan.The strains C_2 (67) 577, DPL-7344-424 and C_2 (69) 1455 acquired from CCRI, Multan were selected as parents for the inheritance studies of bacterial blight. C_2 (67) 577 and C_2 (69) 1455 were resistant to bacterial blight while line DPL-7344-424 was susceptible to this disease. It was observed after screening by applying inoculum of race-18. Seeds of these lines were sown in pots filled with potting mixture containing red soil, farmyard manure and sand with I:2:1 (vol./vol./vol.) ratio placed in glass house in January 2001. Crosses viz., resistant x susceptible, resistant x resistant and susceptible x resistant were made among the two resistant and one susceptible lines in the glass house during March 2001 to develop F_0 seed for growing F_1 generation. The seed thus obtained was then planted in the field to raise F_1 generation alongwith the respective parents next season (2001-02). At flowering backcrosses were made in the field. Parent strains were used to make fresh crosses to have the F_1 hybrid seed of resistant x susceptible, resistant x resistant and susceptible x resistant crosses. Parents, F_1, the seed of selfed F_1 plants to raise F_2 and seed of backcrosses was planted in the pots replicated thrice and placed in the glass house during December 2001-02 to raise P_1, P_2, F_1, F_2, BC_1F_1 and BC_2F_1generations. After emergence seedlings were thinned to a level as required to facilitate a uniform seedling establishment. Initial thinning resulted in 5 seedlings of each parent and F_1 population, 6 seedlings of F_2 population and 4 seedlings of each BC_1 F_1 and BC_2 F_1 populations per pot. The total number of plants used for recording the data in glass house for parents and all other generations are given in Table 2. Plants were inoculated with the X*anthomonas campestris* pv *malvacearum* and evaluated at the true leaf stage. Seedlings were inoculated with inoculum source using the Toothpick Scratch method (Bird, 1986). At adult plant stage, leaves were inoculated and evaluated for the adult plant leaf growth stage.

The race-18 of *Xanthomonas campestris* pv *malvacearum* was cultured at 25^0C on agar medium. Inoculum was prepared from 8-10 days old cultures by placing a small portion of the bacterial growth inside a small glass vial containing sterile water. Plants were evaluated for disease reaction for 15-20 days after inoculation depending upon disease development. Disease expression was scored by the scale 1 (immunity) to 10 (fully susceptible) for bacterial blight as proposed by Bird & Hadley (1958). In the present studies, 1 to 3 was considered as resistant and 4 to 10 as susceptible.

Disease reactions of seedling stage and adult stage leaves within a single plant were examined and highest disease grade was recorded to represent the disease reaction for that plant tissue. Data for disease reactions and disease severity on the plants was recorded. More than one reading of disease severity was collected for disease after regular intervals. The number of individuals falling into resistant and susceptible classes of bacterial blight and the observed reaction alongwith segregation ratios in the F_1, F_2, and back cross populations were tested for goodness of fit using the Chi-square statistics.

Results and Discussion

Average disease severity and disease appearance data for bacterial blight of all generations from the parents and three different crosses (F_1, F_2, and backcrosses) were recorded (Table 1). The results showed that C_2 (67) 577 was resistant to *Xanthomonas campestris* pv *malvacearum* with an average disease index of 1.67 at seedling and 2.13 at adult plant stage. In DPL-7340-424 plants were susceptible with an average disease index of 7.08 and 6.92 at seedling and adult plant stage respectively (Table 1). The variety C_2 (69) 1455 showed average disease severity of 2.13 and 2.16 at seedling and adult plant stage respectively and confirmed as resistant (Table 1). The cross wise results of disease reaction are described below.

Table 1. Disease grade means of seedling and adult plant stage of parents, F_1, F_2, BC_1, and BC_2 in three different cross combinations and repeats inoculated with *Xanthomonas campestris* pv *malvacearum*.

Parents	Seedling stage				Adult stage			
	R_1	R_2	R_3	Mean	R_1	R_2	R_3	Mean
A(R) C_2 (67) 577	1.75	1.67	1.38	**1.67**	2.13	2.00	2.35	**2.13**
B (S) DPL-7340-424	6.75	7.25	7.25	**7.08**	6.25	7.63	6.88	**6.92**
C(R) C_2 (69) 1455	2.00	2.13	2.25	**2.13**	2.00	2.00	2.50	**2.16**
F_1Populations								
A x B F_1(R x S)	1.90	1.90	1.50	**1.77**	2.00	1.80	1.50	**1.77**
A x C F_1(R x R)	1.30	1.20	1.33	**1.28**	1.20	1.25	1.43	**1.33**
B x A F_1 (S x R)	2.20	2.09	2.09	**2.13**	1.90	1.90	210	**1.99**
F_2Populations								
F_2(R x S)	3.99	3.50	4.03	**3.81**	3.13	3.12	3.33	**3.19**
F_2(R x R)	1.72	1.61	1.67	**1.67**	1.64	1.53	1.58	**1.58**
F_2(S x R)	4.42	3.86	4.20	**4.16**	3.31	3.14	3.61	**3.35**
Back crosses								
(R x S) x R, BC_1	2.04	2.04	2.00	**2.03**	1.96	2.04	1.96	**1.99**
(R x S) x S, BC_2	4.50	4.96	4.21	**4.56**	4.19	4.32	3.67	**4.06**
(R x R) x R, BC_1	1.80	1.96	1.71	**1.82**	2.22	1.88	1.79	**1.96**
(R x R) x R, BC_2	1.72	1.80	1.60	**1.71**	1.71	1.65	1.95	**1.77**
(S x R) x S, BC_1	2.08	2.00	2.12	**2.07**	2.04	2.05	1.88	**1.99**
(S x R) x R, BC_2	4.52	5.32	4.12	**4.65**	4.38	5.26	4.00	**4.55**

Table 2. Segregation for seedling and adult plant stage disease reactions to *Xanthomonas campestris* pv *malvacearum* in parents, F_1, F_2, BC_1, and BC_2 generations.

Parent/cross/ generations	Exp Seg.	Seedling plant stage				Adult plant stage			
		Observed segregation				Observed segregation			
		Res.	Sus.	χ^2	P	Res.	Sus.	χ^2	P
A. C_2 (67) 577	R	24	0	-	-	22	0	-	-
B. DPL-7344-424	S	1	23	-	-	2	22	-	-
C. C_2 (69) 1455	R	24	0	-	-	24	0	-	-
A x B									
F_1	R	30	0	-	-	25	0	-	-
BC_1 (F_1x A)	R	75	0	-	-	70	0	-	-
BC_2 (F_1 x B)	1:1	37	36	0.136	0.750-0.500	34	30	0.250	0.750-0.500
F_2	3:1	168	70	2.471	0.250-0.100	167	65	1.046	0.500-0.250
A x C									
F_1	R	29	0	-	-	25	0	-	-
BC_1 (F_1 x A)	R	74	0	-	-	72	0	-	-
BC_2 (F_1x C)	R	75	0	-	-	70	0	-	-
F_2	R	242	0	-	-	231	0	-	-
B x A									
F_1	R	32	0	-	-	30	0	-	-
BC_1 (F_1 x A)	R	70	5	-	-	67	2	-	-
BC_2 (F_1 x B)	1:1	36	39	0.120	0.750-0.500	35	36	0.014	0.900-0.750
F_2	3:1	173	70	1.877	0.250-0.100	165	65	1.305	0.500-0.250

Res. = Resistant, Exp. Seg. = Expected segregation, Sus. = Susceptible, BC = Backcross, χ^2 = Chi-square, P = Probability

1. C_2 (67) 577 x DPL-7340-424 (resistant x susceptible) (A x B): Analysis of the variance of the data indicated significant differences among the parental, BC_1, BC_2 and F_2 populations for both seedling and adult plant stage inoculated with race-18 of *Xanthomonas campestris* pv *malvacearum*. F_2 disease grade frequency distribution was observed in both seedling and adult plant stage. Parental disease grade and F_2 distributions indicated two classes of disease reactions, with grade 1 to 3 being resistant and grade 4 to 10 as susceptible. A chi-square test for homogeneity indicated that replications were homogeneous; subsequently they were pooled (Wallace & El-Zik, 1989). The F_1 plants were resistant but the F_2 population segregated into 3:1 (resistant: susceptible) phenotypic ratios for both seedlings and adult plant stages (Table 2). When F_1 backcrossed to the susceptible parent DPL-7340-424, the population segregated into 1:1 ratio (resistant: susceptible) for both seedling and adult plant stages (Table 2).

The disease grade frequency distributions for the cross C_2 (67) 577 x DPL-7340-424 (resistant x susceptible) indicated a single gene difference with complete dominance for resistance to the race-18 in the resistant parent C_2 (67) 577. The appearance of single gene inheritance, however, conflicts with the reported multigene resistance in the resistant cotton parent Tamcot CAMD-E (Bird, 1974).

2. DPL-7340-424 x C_2 (67) 577 (susceptible x resistant) (B x A): The strain DPL-7340-424 was susceptible to race-18 and C_2 (67) 577 were resistant. Bimodal disease grade frequency distribution was obtained when seedling and adult plant stage leaves of F_2 populations were inoculated with the race-18. Disease grade frequency distributions indicated a resistant and susceptible class represented by grade 1 to 3 and 4 to 10 respectively. A chi-square test for goodness of fit to a 3:1 (resistant: susceptible) phenotypic ratios having a probability values between 0.250-0.100 good fit at seedling plant stage. Adult plant stage disease grade for race-18 also showed a good fit to 3:1

ratio. The segregation of BC_2 population showed poor fit to the expected 1: 1 (resistant: susceptible) ratio (Prob. 0.750-0.500) at seedling whereas good fit at adult plant stage (prob. 0.900-0.750). Single gene inheritance of resistance to the race-18 was also indicated in the C_2 (67) 577 when crossed as male with the susceptible DPL-7340-424.

3. C_2 (67) 577 X C_2 (69) 1455 (resistant x resistant): Parental cultivars C_2 (67) 577 and C_2 (69) 1455 were resistant to the race-18 in reactions of both at seedling and adult plant stage with no significant differences among parents and backcrosses. Disease grades were slightly higher for C_2 (69) 1455 compared to C_2 (67) 577 for both seedling and adult plant stage. No segregation for resistance to the race-18 was observed in this cross both in F_2 population and back cross progenies (Table 2).

Over all it was observed that the age of the leaves at seedling and adult plant stage appeared to influence the degree of disease expression. In general, younger leaves at seedling stage of susceptible plants scored higher disease grading scale than adult plant stage leaves.

The monogenic inheritance of resistance indicates that pedigree breeding would be adequate for transferring the resistance in the susceptible genotypes. Both seedling and adult plant stage F_2 and back cross disease grade distributions clearly indicated a monogenic type of inheritance of resistance in both (resistant x susceptible) and (susceptible x resistant) crosses. All the plants were resistant in F_1, F_2 and back cross progenies in cross between resistant x resistant showings that the gene for resistance is the same in both the parents. Conflicting evidence for a basic mechanism controlling resistance to bacterial blight in different plant parts and at different stages of plant growth have been reported (Arnold, 1963; Arnold & Brown, 1968). Arnold & Brown (1968) suggested that screening for resistance should be conducted at several different stages of plant growth.

Conclusion

Seedling and adult stage disease grade in present study were positively correlated, and monogenic inheritance was observed in both seedling and adult stage of growth. These results support the concept of common basic mechanism controlling the resistance in the two different stages of plant growth and suggest that selection for resistance can be accomplished in either growth stage. Evaluation for resistance at both the seedling and adult stages would give an added measure of assurance to correctly identifying resistant genotypes.

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