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New Gene Combinations Conferring Resistance to the Bacterial Blight Disease of Cotton

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ABSTRACT

F_2 plants comprising different gene combinations were evaluated for cotton bacterial blight resistance in the field of the Gezira Research Station and the greenhouse of the Plant Pathology Centre of the University of Gezira, Neshashiba, Wad Medani, during the autumn of 2002. Results generally showed significant differences between different gene combinations in resistance to the new race of the bacterium *Xanthomonas campestris pv. malvacearum*. Leaf disease grades ranged from 4 to 6. Most of the plants fell into grade 5. Genotype 12, with expected gene combination of ($B_2B_3B_6B_7B_9B_{12}$), expressed the highest level of resistance with a mean disease grade of 4, while genotypes 7, 11 and 15 showed the lowest level of resistance with a mean disease grade of 6. The results indicated the importance of the genetic background $B_2B_3B_7$ in improving resistance to both the old and the new races when one of the following major genes B_4, B_6, B_9، and B_{12} is incorporated. In such background, resistance level improves as the number of genes increases. Therefore, the commercial resistant cultivars having B_2B_6 genetic background to the old race of the bacterium can be replaced by the genetic background involving $B_2B_3B_7$ in addition to one of the four major genes mentioned above in order to protect the cotton crop. Genotypes 5, 6, 12, and 19 were recommended for further improvement as they expressed relatively high level of resistance to the pathogen.

INTRODUCTION

Cotton production in Sudan has many constraints, among these are insects, weeds and diseases, causing potential loss in yield and fiber quality. Bacterial blight of cotton, caused by *Xanthomonas compestris pv malvacearum* (Smith) Dye, is a major foliar disease and one of the most studied diseases for the past 50 years. Bacterial blight occurs in most cotton producing countries of the world and it is an important disease, especially in countries which experience periods of wind and rain during the growing season such as Sudan. Although, complete crop failure due to the disease was not reported, reduction in seed cotton of 14-21% has been recorded (El Nur, 1970).

Control measures of the disease adopted included a combination of resistant cultivars, chemicals (mainly seed dressing with fungicides), cultural practices and legislative measures. Complete elimination of the disease, however, can be achieved only by the cultivation of varieties genetically immune to the disease, which is considered to be the most economical and effective means of controlling bacterial blight. Breeding for resistant varieties started since the disease was first reported. In the 1940s and early 1950s, a program of backcrossing to adapted local susceptible varieties of Egyptian and Upland cotton was undertaken. The first commercial variety of long staple cotton (*G.barbadense*) was Bar X LI which became the most important variety for irrigated schemes in the late 1950s and 1960s (Innes .(1974). Later, Barakat variety with B_2B_6 gene combination replaced Bar X LI in the 1970s (Siddig, 1973). Then the resistance genes were transferred to Acala 4-42 to develop Barac (67) B, which is a widely grown variety in Gezira and several production areas in the Sudan: In the mid 1950s, the old race (Pre-Barakat) was controlled by releasing cultivars having B2B6 resistance genes. The relaxation of clean-up campaigns and the application of legislative measures have led to the appearance of a new race (Post-Barakat) that has broken down the resistance conferred by B_2B_6 gene combinations.

On this background, the current study is carried out to compare different gene combinations in order to identify the best one for resistance to the prevailing bacterium races as well as for the future races.

The objectives also include the determination of the mode of inheritance for resistance to bacterial blight.

MATERIALS AND METHODS

Field as well as greenhouse experiments, were carried out during 2002 cotton growing season to study the magnitude of bacterial blight resistance (Post-Barakat race) of 21 different cotton genotypes (treatments). The full description of the treatments and their expected gene combinations are given in Table l. The genetic material for this study was kindly provided by Dr. Ahmed Mohamed Mustafa, Cotton Research Program, Agricultural Research Corporation (ARC) of the Sudan.

Table l. Treatments description of the expected gene combinations of the cotton F_l plants used in the experiments, Gezira Research Station and University of Gezira, 2002.

Tr. no. Treatments description (genotypes)	Expected gene combination in the F_1
1. (In 15/Reba) F_4(1n I0/Reba)D2156F_3	B_2 $B_3B_7B_9$
2. (Reba/B67) F_5/(Reba/ln 15) D215F_3	B_2 $B_3B_6B_7$
3.(B90/sh. coll) F_4(1nt 15/ Reba)F_3	B_2 $B_3B_6B_7$ B_9
4. (Reba/Int 10)F_3/(Int 10/Reba) D50F_3	B_2 $B_3B_7B_9$
5. (B 67/1n 10) F_3/Reba/In 15)D215F_3	B_2 $B_3B_6B_7$ B_9
6. (In10/Reba)F_3/(Reba/In10) D50F_3	B_2 $B_3B_7B_9$
7. (In15/Reba)F_4/(In5/AH)/(CACHUS-2-86)F_3	B_2 $B_3B_6B_7$ B_9
8. (B90/sh. Coll)F_4/(TC44/TC32)F_3	B_2 $B_3B_6B_7$ B_{12}
9. (Reba/lnt 10) /(CABCHUS-2-86F_3/(TC44/TC32)F_3	B_2 $B_3B_7B_9$ B_{12}
10. (TC32/B90)F_2/B90	$B_2B_6B_{12}$
11. {(TC20/TC39)F_1(TC39/TC32)f_1}f_3/ {(Reba/Int10)	B_2 $B_3B_4B_7$ B_9 B_{12}
12. {(AH/TC32)F_l/Bar} F_3/(Reba/Int10)F_1/D5}F_3	B_2 $B_3B_6B_7$ B_9 B_{12}
13. (TC44/TC32)F_3/B67	B_2 $B_3B_6B_7B_{12}$
14. (AM/1nt 15)F_4/ (Reba/1nt15)F_4	B_2 $B_3B_6B_7$ B_9
15. {(AH/B67)F_l/BLCABPD8GS-1-90}F_3/(Int 10/Reba)F_3	B_2 $B_3B_6B_7$ B_9
16. (B67/SP 37) F_3/{Reba/In 10)F_1/D2156}F_3	B_2 $B_3B_6B_7$ B_9
17. (Int 15/Reba) F_3/{Reba/In 10) F_1/D2156} F_3	B_2 B_3B_7 B_9
18. { (TXCAHU8-2-81/Reba)F3t 15/Reba) F_3}/{Reba/Int 10) F_1/D2156} F_3	B_2 B_3B_7 B_9
19. { (TXCAHU8-2-81/Reba)F_3 }/Reba/TXCAHU8-2-81)F_1/D50) }F_3	B_2 B_3B_7 B_9
20. (Int 15/Reba) F_4/{In 5/AH)/CAPCHUS-286)F_3	B_2 $B_3B_6B_7$ B_9
21. . (TC20/AH)F_1/AH	B_2 $B_3B_6B_7$

Field experiment

This study was conducted in the field of Gezira Research Station (GRS), ARC, wad Medani, Sudan (latitude 14^0 24' and longitude 33^0 29'E), during July-October, 2002. The experiment was laid out in a randomized complete block design with 3 replications and 21 treatments. These were planted in 6m long rows with 0.8m spacing between rows and 0.5m between holes. Cultural practices followed were those recommended for cotton production. The sowing date was the 20th of July, 2002.

Greenhouse experiment

The seeds were sown in plastic pots, 30 x 20 cm, and kept in the greenhouse of the Plant Pathology Center (PPC), University of Gezira (U. of G.), El Neshashiba, Wad Medani, during September November, 2002. The seeds of the 21 treatments, each representing a gene combination, were sown on a 2:1 soil mixture of silt and sand. The treatments were arranged in a completely randomized design with three replications.

Inoculum preparation, Inoculation and scoring of disease severity

Nutrient glucose agar medium (NGA) was used for isolation, purification, propagation and storage of the bacteria. Barakat 90, a susceptible cotton variety, was used for isolating the bacterium. 48-72 hours old cultures were washed with sterile distilled water in 500ml flasks. The flasks were agitated and the bacterial suspension was diluted with sterile water to produce an inoculum density of approx- imately 1.0 $x10^6$ bacteria/ml. In the greenhouse experiment, both cotyledon and true leaves were inoculated using the tooth pick scratch method (Bird, 1982), by making a scratch on the underside of a seven-day old cotyledon and on the underside of six-week old plant true leaves, dipping the tooth pick in the inoculum after each scratch.

Inoculum preparation from infected trash

Five pounds of infected dried leaves were soaked in 40 gallons of water for 2 hours and then continuously crushed and stirred. Then the suspension is strained and used immediately. Knapsack sprayers were used for field spraying. Six-week old plant true leaves were inoculated

using spraying. Inoculation was done in the morning when stomata were fully open and spraying was directed to the lower surface of the leaves.

Grading system

Plants were assessed for resistance three to four weeks after inoculation. The grading system developed by Bird and Hadley (1958) was used for disease assessment in the greenhouse experiment and that developed by Knight (1946) was used in the field experiment. For the first grading system, the grades range from I to 10, with 1 representing immunity and 10 full susceptibility. While for the second grading system the grades ranged from 0 to 10, with 0 representing immunity and 10 full susceptibility.

RESULTS AND DISCUSSION

The field results indicated significant differences in leaf disease grades between the different genotypes. The mean disease grades ranged from 4 to 6 for the F_2 plants grown in the field (Table 2). Most of the F_2 plants (66%) scored a mean disease grade of 5. F_2 plants of genotype 12, which was expected to have $B_2B_3B_6B_7B_9B_{12}$ gene combination, expressed the highest level of resistance with a mean of 4 and a range of 2 to 7. Similarly, genotype 5 with expected gene combination of $B_2B_3B_6B_7B_9$, scored a mean disease grade of 4.3, and a range of 2 to 7. The resistance in the first genotype was better than the second one. This result showed that B_{12} had a great effect on enhancing the resistance when it was added to the genetic background $B_2B_3B_7$, or it might be that the absence of B_{12} in the second genotype reduced the resistance. Both genotypes 6 and 19, which were expected to have the same gene combination of $B_2B_3B_7B_9$, scored the same mean disease grade of 4.7, and a range of 2 to 7 and 3 to 7, respectively.

These results indicated that the best gene combination conferring high resistance to the new race of the bacterium was $B_2B_3B_6B_7$ B_9B_{12}‘ followed by $B_2B_3B_7B_9$ gene combinations. Both genotypes had B_3, B_7 and B_9 genes. The first gene combination gave better mean disease grade than the second one. This could be due to the presence of B_6‘ B_{12} or both. These results agreed in partial with those of Follin *et al.* (1988) and Wallace and El-zik (1989) who reported that resistance to VHI in S295

was controlled by a single gene, designated B_{12}, with complete dominance for resistance. The results also agreed with that of Innes (1964) who found that B_2B_9 conferred resistance as effective as B_2B_6 or $B_2B_3B_6$, consequently B_9 might have enhanced resistance to the new race of the bacterium. Innes (1964) stated that B_3 and B_6 were additive and when combined they conferred a resistance better than that given by B_6 alone.

Table 2. Mean and range of disease grades of 21 cotton genotypes evaluated for bacterial blight resistance.

Tr. no.	Expected gene combinations	Field		Greenhouse			
				Cotyledons		Leaves	
		Mean Disease grade	Range	Mean Disease grade	Range	Mean Disease grade	Range
1.	B_2 $B_3B_7B_9$	5.0 abcd	3-7	5.3 bc	2-8	2.7 c	1-5
2.	B_2 $B_3B_6B_7$	5.0 abcd	4-7	5.3 bc	3-8	2.7 c	2-4
3.	B_2 $B_3B_6B_7$ B_9	5.3 abc	3-8	4.7 c	3-8	3.7 abc	1-7
4.	B_2 $B_3B_7B_9$	5.0 abcd	4-8	5.3 bc	4-8	3.3 bc	3-5
5.	B_2 $B_3B_6B_7$ B_9	4.3 bc	2-7	6.3 abc	6-8	4.0 abc	2-5
6.	B_2 $B_3B_7B_9$	4.7 bcd	2-7	6.0 bc	4-7	4.0 abc	1-6
7.	B_2 $B_3B_6B_7$ B_9	6.0 a	2-10	5.0 bc	3-9	3.3 bc	2-5
8.	B_2 $B_3B_6B_7$ B_{12}	5.7 ab	3-8	6.7 ab	3-10	4.3 ab	3-6
9.	B_2 $B_3B_7B_9$ B_{12}	5.7 ab	2-10	7.7 a	5-10	4.3 ab	2-6
10.	$B_2B_6B_{12}$	5.7 ab	5-9	5.7 bc	5-7	4.3 ab	2-6
11.	B_2 $B_3B_4B_7$ B_9 B_{12}	6.0 a	3-10	5.0 bc	3-7	3.3 bc	1-6
12.	B_2 $B_3B_6B_7$ B_9 B_{12}	4.0 d	2-7	5.0 bc	3-7	3.7 abc	1-4
13.	B_2 $B_3B_6B_7B_{12}$	5.7 ab	5-8	5.3 bc	5-7	3.7 abc	2-7
14.	B_2 $B_3B_6B_7$ B_9	5.0 abcd	4-7	6.3 abc	5-8	5.0 a	4-6
15.	B_2 $B_3B_6B_7$ B_9	6.0 a	4-10	6.0 bc	5-7	4.0 abc	2-6
16.	B_2 $B_3B_6B_7$ B_9	5.0 abcd	3-7	6.0 bc	5-9	4.0 abc	3-5
17.	B_2 B_3B_7 B_9	5.7 ab	3-7	5.0 bc	3-10	3.3 bc	2-6
18.	B_2 B_3B_7 B_9	5.0 abcd	3-7	5.3 bc	2-10	4.3 ab	2-7
19.	B_2 B_3B_7 B_9	4.7 bcd	3-7	6.3 abc	4-8	3.7 abc	2-8
20.	B_2 $B_3B_6B_7$ B_9	5.0 abcd	2-10	5.3 bc	4-8	2.7 c	2-5
21.	B_2 $B_3B_6B_7$	5.0 abcd	3-8	5.0 bc	2-8	3.0 bc	1-6

*Disease grades range from I to 10, with I representing immunity and 10 full susceptibility.

Means followed by the same letter(s) are not significantly different at the probability level of 0.05 according to Duncan's Multiple Range Test (DNRT).

Genotype 7, with an expected gene combination of $B_2B_3B_6B_7$ B_9، gave the highest mean disease grade (6) and the largest range (2-10). Though the highest and lowest resistance was conferred by genotypes 12 and 7, respectively, both genotypes had the same genetic combination with the absence of B_{12} in genotype 7. This finding strongly confirmed the fact that B_{12} enhanced resistance to the new bacterial blight race.

The B_4 major gene appeared to have no big effect on resistance to the new race of bacterium because when B_4 replaced B_6 gene in the genotype number 11, the plants scored a mean disease grade of 6.0. The gene B_4 in the Empire background acts as a strong gene of a large
effect (Bird, 1974) and confers a greater degree of resistance than the gene combinations B_2B_6, B_2B_3 or $B_2B_3B_7$ (El-zik and Bird, 1970). This result emphasized the importance of the effect of genetic background on the expression of major genes for bacterial blight resistance. This importance was also emphasized by the difference in the effect of B_7 when transferred to Wilds sus 16/1 and Acala 4-42، both susceptible Uplands (Hughes, 1961).

When the treatments were arranged into groups having the same genotypes, the $B_2B_3B_7B_9B_{12}$ gene combination showed the highest level of resistance. It was observed that adding B_4, B_6, B_9 or B_{12} to the genetic background $B_2B_3B_7$ improved resistance, however, B_9 or B_{12} acted better than B_4 and B_6 on the same genetic background. As Innes (1964)reported these major genes might be acting additively and if this was true, one would expect durable resistance persisting for a longer period of time since it would be difficult for the pathogen to break down the resistance of more than two genes at a time.

Both cotyledons and true leaves were evaluated in the greenhouse. Cotyledons mean disease grades ranged from 4.7 to 7.7 for the F_2 plants (Table 2) with 90 percent of the plants falling into grades 5 and 6. Genotype 3 with .the expected $B_2B_3B_6B_7B_9$ gene combination showed the highest resistance with mean disease grade of 4.7, while genotype 9 with the expected gene combination of $B_2B_3B_7B_9B_{12}$ showed the highest disease grade of 7.7. The result of these two genotypes indicated the importance ofB6 for resistance on the genetic

background $B_2B_3B_7B_9$ to the Post-Barakat race of bacterium. The major genes for resistance, B_3 and B_7, which were introduced from the multi-adversity resistance (MAR) program from the USA might have contributed positively in conferring resistance to the new Post-Barakat race of bacterium in the presence of the B_6 gene.

True leaves mean disease grades ranged from 2.7 to 5.0 for the F_2 plants grown in the greenhouse (Table 2). The true leaf grades, however, were lower than cotyledon disease grades. Eighty percent of the plants fell into grades 3 and 4. The differences for disease reaction of true leaves were very small compared to those for cotyledons. The mature true leaves were less susceptible than cotyledons, the latter were more sensitive to the pathogen. Massey (1934) showed that plant tissues became less susceptible with age and that senescence conferred immunity.

Genotypes 1, 2 and 20, with expected gene combinations (B_2B_3 B_7B_9), ($B_2B_3B_6B_7$) and ($B_2B_3B_6B_7B_9$), respectively, displayed the highest level of resistance of the same disease grade of 2.7. This result was different from that observed in the field and in the cotyledon stage in the greenhouse where the three genotypes scored a disease grade of 5.0. Genotype 14 with the same expected gene combination of ($B_2B_3B_6B_7B_9$) as that of 20, expressed the lowest level of resistance of 5.0 Similar results were observed for this genotype under field conditions and in the cotyledon stage in the greenhouse. The low average disease grades resulting from the stage of true leaves, however, suggested that the greenhouse conditions were not favourable for disease progress.

When comparing true leaves in the field with true leaves in the greenhouse, the combined analysis of variance (Table 3) showed significant mean squares for location, genotypes and the interaction of location x genotypes. This could be attributed to the fact that, the field experiment succeeded in differentiating between the genotypes into resistant and susceptible genotypes because of the favourable environment in the field for the growth of the pathogen. The season witnessed high rains during the period July-October. The rain distribution during the season was high enough to maintain a high relative humidity

in the field that favoured the bacterial blight infection and spread of disease throughout the field while the environmental conditions in the greenhouse probably might not be proper for infection. El Nur (1970) reported that climatic conditions strongly influenced infection and the spread of the disease, both within an individual plant and in a crop, as well as the severity of symptoms.

Table 3. Mean squares for location, genotypes and their interaction bacterial blight disease grades for 21 cotton genotypes, Wad Medan2002.

SOV	DF	Mean square		
		True leaves (Field x true Leaves (greenhouse)	True leaves (filed) x Cotyledons (greenhouse)	(True leaves(green-house) x cotyledons (greenhouse
Location (L)	1	73.75**	6.05	122.03**
Genotypes (C)	20	1.09*	1.23	2.20*
L x C	20	1.01*	1.26	0.57

*, ** Significant at the 0.05 and 0.01 level of probability, respectively.

The results of the greenhouse experiment disagreed with those obtained by Innes (1961) who showed that greenhouse tests were carried out successfully both in and out of season and had enabled selection to be made for progenies homozygous for B_2B_6 grown in the field. Such discrepancies could be attributed to the properly managed and controlled environmental conditions used in Innes experiment. while in the present study, the relative humidity was not optimum (low) for disease progress. In the greenhouse, fully grown leaves test showed that most of the plants appeared resistant. The field and the greenhouse tests were really needed for proper evaluation of the genotypes for resistance.

In comparing true leaves (field) with cotyledons (greenhouse), the

3 sources of variation showed non-significant mean squares which could be explained as plants in cotyledon stage might find environmental conditions for the disease development during the period of September better than in the true leaves stage in the greenhouse Therefore, they showed a high rate of disease similar to that in the field, thus both true

leaves in the field and cotyledons in the green- house responded similarly to the new race of bacterium.

Even within the greenhouse environmental conditions, the combined analysis showed significant differences for location and genotypes when comparing true leaves with cotyledon. Such finding were attributed to environmental conditions (low relative humidity and temperature) changes. Also, it might be a physiological change in the mature plants, and therefore, true leaves showed high resistance compared to cotyledons. Genotypes behaved similarly in both stages (full leaves and cotyledons) in the same environment, thus, there was no significant difference in the interaction effect.

The Chi-square test was used to determine goodness of fit of the data from the field experiment to the proposed genetic models for the mode of inheritance of resistance to the new race of bacterium. One gene model (Table 4) was used, but no clear cut genetical ratios were obtained for most genotypes, with the exception of the genotypes 5,6,12,18 and 19 which fit closely the ratio of 3 resistant: I susceptible. The resistance in these genotypes might be controlled by one single dominant gene from the major genes B_3, B_7, B_9 or B_{12}. Innes (1964) reported that four families, BA 1-4/62, each derived from selfed F_1 plant (Bar 14/11 (B_1) x Bar 14/53 (B_6) were screened for resistance. The data were found to fit more closely the 3:1 ratio.

The F_2 data from the field experiment did not fit a 2-gene model، for the two independent genes, with a ratio of 9:3:3:1, or two dependent genes with the ratios of 9:4:3, 12:3:1, 15:1 and 13:3. However، some genotypes (1,2, 14, 17, 20 and 21) fit the ratio of 9 resistant: 7 susceptible (Table 5). This ratio suggested an epistatic expression of the 9 :3:3:1 which, in turn, indicated two pairs of genes with duplicate recessive epistasis. Quantitative analysis to bacterial blight indicated additive, dominance, and epistatic gene action (Innes *et al*., 1974؛ Wallace and El-zik, 1990), suggesting that the resistance might be controlled by 2 of B_2, or B_3, B_6, B_7, B_9 or B_{12} genes. Innes (1964) in a diallel set of genotypes made between seven Bar strains obtained an excellent fit to a 9:4:3 ratio for bacterial blight resistance. This ratio is expected for the segregation

of major dominant and recessive genes، which individually confer intermediate resistance and together are additive, giving high resistance.

Table 4. Reaction of field grown, F_2 plants (true leaves) of 21 cotton genotypes, evaluated for bacterial blight resistance, with Chi-square goodness of fit to a 9R: 7S ratio and P value, GRS, Wad Medani، Sudan, 2002.

Tr. no.	No. of plant observed		X^2	P
	R	S		
1	118	79	22.0	-
2	72	51	16.0	-
3	54	68	59.0	-
4	174	94	18.0	-
5	168	48	0.8	0.99 >0.30
6	171	66	0.1	0.30
7	49	115	177.0	-
8	47	107	166.0	-
9	59	123	171.0	-
10	36	104	198.0	-
II	89	206	313.0	0.30 >0.05
12	95	42	2.4	-
13	54	122	184.0	-
14	45	49	38.0	-
15	36	101	175.0	-
16	149	87	19.0	-
17	50	49	31.0	-
18	117	52	0.3	0.30 >0.05
19	153	70	4.0	0.05 >0.01
20	121	108	56.0	-
21	105	79	30.0	-

R = Resistant (1-5 grades), S = Susceptible (6-10 grades)

Table 5. Reaction of field grown, F_2 plants (true leaves) of 21 cotton genotypes, evaluated for bacterial blight resistance, with Chi-square goodness of fit to a 9R: 7S ratio and P value, GRS, Wad Medani, Sudan, 2002.

Tr. no.	No. of plants observed		X^2	P	
	R	S			
1	118	79	1.32	0.30	>0.20
2	72	51	0.21	0.99	>0. 5
3	54	68	7.13		-
4	174	94	15.97		-
5	168	48	39.85		-
6	171	66	24.73		-
7	49	115	49.01		-
8	47	107	40.04		-
9	59	123	41.23		-
10	36	104	56.75		-
II	89	206	89.59		-
12	95	42	9.61		-
13	54	122	46.75		-
14	45	49	2.05	0.30	>0. 5
15	36	101	49.90		-
16	149	87	44.21		-
17	50	49	1.02	0.99	>0. 30
18	117	52	11.64		-
19	153	70	14.27		-
20	121	108	1.35	0.30	>0. 20
21	105	79	0.09	0.99	>0. 30

R = Resistant (1-5 grades), S = Susceptible (6-10grades).

The cotton breeding program of the Agricultural Research Corporation of the Sudan will pursue its historical goal of developing adapted, high yielding cultivars of cotton with stable type of resistance to the virulent races of the bacterial blight disease. In conjunction with such pursuit, the current study suggested the use of the genotypes number 5,6,12 and 19 for further genetic improvement because they expressed relatively high level of field resistance to the pathogen through a combination of different resistance genes. Breeding for horizontal resistance would be the key to the different control measures suggested to reduce yield losses caused by the bacterial blight.

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