Inheritance of Tobacco Etch Virus Resistance Found in *Nicotiana tabacum* Cultivar Havana 307

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ABSTRACT

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The inheritance of resistance to tobacco etch virus (TEV) found in *Nicotiana tabacum* cv. Havana 307 (HA 307) was investigated by analysis of disease severity data from sexually derived generations of HA 307 crossed with TEV-susceptible burley tobacco cvs. Kentucky 14 and Jaraiz 1. Parental, F_1, F_2, and both backcross generations (BC_1, BC_2) were grown for each cross in randomized complete block designs with three replicates. Evaluations for virus reaction were performed under greenhouse and field conditions. Generation means analysis indicated that additivity (lack of dominance) was the predominant genetic effect with no evidence of epistasis. A simple Mendelian gene model did not fit the data, but control of TEV resistance is postulated to be due to a few genes with additive effects based on the high frequency of resistant genotypes observed in F_2 generations. Selection for TEV resistance at the haploid level was effective compared with selection among doubled haploid lines. Therefore, utilization of haploids in a breeding program for TEV resistance should allow development of completely homozygous resistant lines in a short time.

Additional keywords: haploid breeding

In western North Carolina, burley tobacco (*Nicotiana tabacum* L.) is the most important crop grown, with an estimated state value of $50 million annually. Virus diseases significantly reduce yield and quality of burley tobacco over the entire U.S. region of burley production, which includes Virginia, Kentucky, North Carolina, and Tennessee (6). The most damaging virus diseases in the region are caused by two members of the potyvirus group: tobacco vein mottling virus (TVMV) and tobacco etch virus (TEV) (4,5,12). In 1985, TVMV and TEV caused an estimated loss of $2 million in North Carolina alone (9).

Although virus diseases of tobacco are controlled primarily by resistant cultivars, burley tobacco cultivars resistant to TVMV and TEV have not been available for commercial use until very recently (11). The cultivar Tennessee 86 has been released with resistance to these viruses derived from Virgin A Mutant, or VAM (11). Unfortunately, VAM as a source of resistance has several limitations, such as poor agronomic characteristics and extreme susceptibility to chewing insects (14) and tobacco blue mold (13). Consequently, burley tobacco breeders have searched for alternative sources of resistance that are not associated with deleterious characteristics.

Havana 307 (HA 307), a cigar tobacco, has been reported to be resistant to several viruses, including TEV and TVMV (7). Therefore, a breeding program was initiated in North Carolina to develop burley tobacco germ plasm with virus resistance from this source. The purpose of this study was to determine the types of gene effects involved in the inheritance of resistance to TEV in HA 307 when crossed with commercial burley tobacco cultivars. The feasibility of selecting TEV-resistant genotypes at the haploid level was also evaluated and compared with the efficiency of selection among doubled haploid (DH) lines.

MATERIALS AND METHODS

Greenhouse experiment. HA 307 was crossed with two TEV-susceptible burley tobacco cultivars, Kentucky 14 (Ky 14) and Jaraiz 1. Parental (P_1, P_2), F_1, F_2, and backcross ($F_1 \times P_1 = BC_1$, $F_1 \times P_2 = BC_2$) generations were grown in the greenhouse and evaluated for TEV resistance. Progeny from each pair of parents were placed in a randomized complete block design with three replicates. Table 1 shows the total

Table 1. Disease index means of parental (P_1, P_2), F_1, and F_2, and backcross (BC_1, BC_2) generations for reaction to tobacco etch virus of two crosses of burley tobacco under greenhouse and field conditions

Entry	Generation[a]	No. of plants[b]	Disease index means[c]	
			Greenhouse	Field
Cross 1				
Ky 14[d]	P_1	24	4.0	4.6
Havana 307	P_2	24	1.0	0.7
Ky 14 × Havana 307	F_1	24	2.5	3.0
(Ky 14 × Havana 307) (s)[e]	F_2	186	2.4	3.1
(Ky 14 × Havana 307) × Ky 14	BC_1	197	3.2	3.8
(Ky 14 × Havana 307) × Havana 307	BC_2	202	1.6	1.8
Cross 2				
Jaraiz 1	P_1	24	4.0	5.0
Havana 307	P_2	24	1.4	0.9
Jaraiz 1 × Havana 307	F_1	24	2.5	3.2
(Jaraiz 1 × Havana 307) (s)	F_2	144	2.5	3.3
(Jaraiz 1 × Havana 307) × Jaraiz 1	BC_1	173	3.6	3.9
(Jaraiz 1 × Havana 307) × Havana 307	BC_2	168	2.2	2.1

[a] P_1 = parent 1 (susceptible), P_2 = parent 2 (resistant), $BC_1 = (F_1 \times P_1)$, $BC_2 = (F_1 \times P_2)$.
[b] Number of plants in the greenhouse. There were 66 plants in each generation in field experiments.
[c] Disease index scale: 0 = no symptoms, 1 = < 1% leaf surface mottled, 2 = 1–5% mottled, 3 = 6–25% mottled, 4 = 26–50% mottled, 5 = > 50% mottled.
[d] Kentucky 14.
[e] (s) = Self-pollinated.

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number of plants evaluated in each generation. Segregating generations (F_2, BC_1, and BC_2) were represented by larger populations to estimate their means more accurately. Plants of the two crosses were grown 4 wk apart to conserve space and to facilitate data collection. Seeds were sown in plastic pots containing a commercial potting mixture and placed on a greenhouse bench. Greenhouse temperatures ranged from 25 to 32 C. Seedlings were transferred at the two- to three-leaf stage into 5 × 5 cm peat pots containing a 1:1 mixture of sterilized soil and commercial potting mixture. Approximately 1 g of a slow-release fertilizer (14-14-14) was added to each peat pot. Plants were inoculated with TEV when the two oldest leaves reached 5–8 cm in length, usually 3 wk after transplanting.

Isolate NC-19 (6) of TEV was used in all trials. Stock cultures of the virus were maintained in plants of tobacco cultivar Burley 21 grown in isolation to prevent contamination from other viruses. Inoculum was prepared by homogenizing systemically infected leaves in 0.05 M $Na_2HPO_4KH_2PO_4$ buffer, pH 7.2, at the rate of 1 g of tissue/5 ml of buffer. The homogenate was pressed through cheesecloth, and 1 g of 22-μm (600-mesh) Carborundum was added to each 100 ml of inoculum. Inoculations were performed with an airbrush propelled by CO_2 at 10 kg/cm^2 and applied to leaf tips until an area approximately 5 mm in diameter appeared water-soaked. Plants were assessed for reaction to TEV 3–4 wk after inoculation, when virus symptoms were clearly expressed. Virus reaction was rated according to a disease index scale from 0 to 5 in which 0 = no symptoms, 1 = < 1% leaf surface mottled, 2 = 1–5% mottled, 3 = 6–25% mottled, 4 = 26–50% mottled, 5 = > 50% mottled. Indexes 0, 1, and 2 were considered resistant reactions, index 3 was an intermediate disease reaction, and indexes 4 and 5 were susceptible reactions.

Mendelian segregation ratios were calculated from disease severity data using a chi-square goodness of fit test. In addition, the generation means analysis procedure outlined by Mather and Jinks (10) was used to test an additive-dominance genetic model for virus resistance. Each of the six mean disease severity phenotypes per cross (P_1, P_2, F_1, F_2, BC_1, BC_2) can be described in terms of m = the midparent value or the midpoint between the two homozygous parents, $[d]$ = the additive effect of the genes or the sum over loci of all ds that measure the departure of each homozygote from the midparent m, and $[h]$ = the dominance effects of the genes or the sum over loci of all hs that measure the departure of the heterozygote from the midparent. Six equations were available for estimating the model parameters (m, $[d]$, $[h]$), and these were obtained by equating the observed family means to their expectations in terms of the three parameters (10). There were three more equations than unknowns (m, $[d]$, $[h]$); therefore, the estimations of the three parameters were done by a least squares technique. Because the number of individuals varied in each generation, the six means were weighted by the reciprocal of their corresponding variance, as suggested by Mather and Jinks (10). A joint scaling test as proposed by Cavalli (3) was used to test the fit of the additive-dominance model using a chi-square goodness of fit test with 3 df. In addition, individual scaling tests as defined by Mather and Jinks (10) were computed to compare with results of the joint scaling test. The individual scaling tests are: $A = 2\bar{BC}_1 - \bar{P}_1 - \bar{F}_1$, $B = 2\bar{BC}_2 - \bar{P}_2 - \bar{F}_1$, and $C = 4\bar{F}_2 - 2\bar{F}_1 - \bar{P}_1 - \bar{P}_2$, where A, B, and C should equal to zero (as determined by a t test) if the additive-dominance model adequately describes the genetic variance (10).

F_1 hybrids (Ky 14 × HA 307 and Jaraiz 1 × HA 307) were subjected to in vitro anther culture (1) to produce 1,108 androgenic haploids. An array of 56 maternally derived haploids was also produced by pollinating the same F_1 hybrids with *N. africana* Merx. & Butt. using the procedure outlined by Burk et al (2). Androgenic and gynogenic haploids constituted a population of 1,164 genotypes evaluated at the haploid level for resistance to TEV. In addition, a random sample of 83 anther-derived haploids from the cross Jaraiz 1 × HA 307 was subjected to in vitro leaf midvein culture (8) to double chromosome numbers and to obtain completely homozygous DH lines. Frequencies of resistant genotypes in haploid and DH populations were compared to determine if selection for TEV resistance could be performed among haploid plants. Haploids were grown in a growth chamber maintained at 25 C, 18-hr photoperiod, and light intensity of 10 W·m^{-2}·s^{-1}. Haploids and DHs were transferred to a greenhouse at the two- to three-leaf stage and grown in the manner described for seedlings. Parental cultivars were used as controls in all tests.

Mendelian segregation ratios for tobacco mosaic virus (TMV) resistance and for burley phenotype were also determined in the haploid populations. TMV resistance is controlled by a single dominant gene and the burley phenotype, by two recessive genes (15). These two traits were used as markers to ascertain the validity of segregation observed for TEV resistance. In other words, if expected segregation ratios were observed for TMV resistance and burley color, then one could safely assume that segregation for TEV resistance was unbiased and that the methodologies employed in haploid development did not impose selection either in favor of or against any given genotype. The procedure used for inoculating plants with TMV was the same as that used with TEV. A TMV-resistant (hypersensitive) reaction was denoted by development of local lesions.

Field experiments. The same generations from the two crosses used in the greenhouse experiments were evaluated in the field at the Mountain Research Station, Waynesville, NC, in the summer of 1987. The experiment was conducted as a randomized complete block design with three replicates. Each replicate consisted of six plots corresponding to the six entries (P_1, P_2, F_1, F_2, BC_1, BC_2). We also evaluated 100 DH lines derived

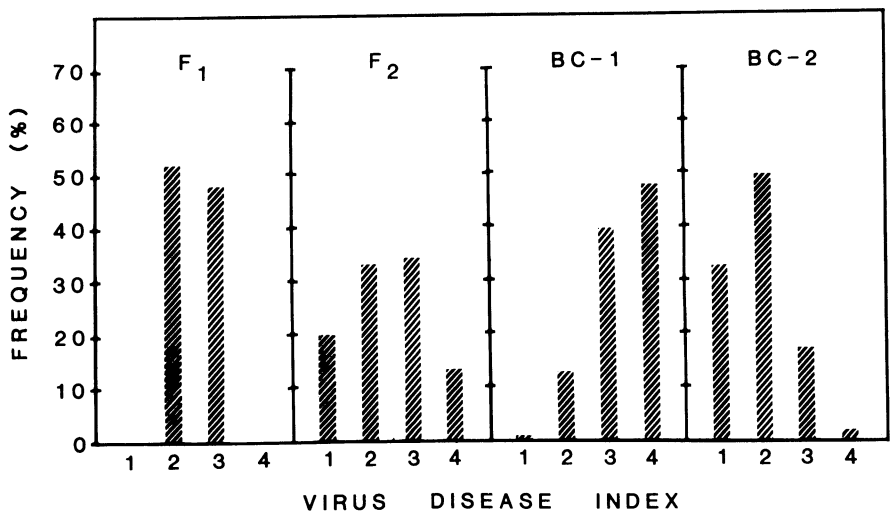

Fig. 1. Frequency distribution of resistant and susceptible genotypes in F_1, F_2, and backcross generations using average disease indexes from two crosses (Ky 14 × Havana 307 and Jaraiz 1 × Havana 307) inoculated with tobacco etch virus in a greenhouse. Virus disease index scale: 0 = no symptoms, 1 = < 1% leaf surface mottled, 2 = 1–5% mottled, 3 = 6–25% mottled, 4 = 26–50% mottled, 5 = > 50% mottled.

from haploid lines (from both crosses) that had been identified as TEV-resistant in the greenhouse tests. The DH lines were planted in a randomized complete block design with three replicates. A correlation coefficient was calculated for virus haploid reaction in the greenhouse and DH reaction in the field. Parental lines HA 307, Ky 14, and Jaraiz 1 were included to serve as controls. Seeds of all genotypes were sown in plant beds on 30 March 1987 and transplanted to the field on 25 May 1987. All field plots consisted of single rows of 22 plants spaced 46 cm apart within the row and 122 cm between rows. Standard cultural practices for burley tobacco were used in every case. Virus inoculations were performed on 23 June 1987 with the same TEV isolate and inoculation procedure used in the greenhouse experiments. Assessments of virus reactions were made on 20 July 1987 using the scale described above. Genetic analyses were conducted as with greenhouse experiments.

RESULTS AND DISCUSSION

Frequency distributions of genotypes into resistant and susceptible classes for F_1, F_2, and backcross generations (BC_1, BC_2) of greenhouse grown plants are shown in Figure 1. Data represent average disease indexes obtained from the two crosses involving resistant HA 307 and susceptible Ky 14 and Jaraiz 1 because distributions within each family were very similar. Individuals in the F_1 generation were classified into two classes (indexes 2 and 3) with approximately equal frequencies, 52 and 48%, respectively. Because all plants in the F_1 generation were genetically identical, this variability must have been due to environmental factors. The F_2 generation followed approximately a normal distribution. Backcross generations to the susceptible parents (BC_1s) were skewed in the direction of susceptibility. Conversely, backcross generations to the resistant parent (BC_2s) were skewed in the direction of resistance. Results from the field experiment were very similar to those obtained in the greenhouse with the exception of more severe symptom expression in the susceptible parents under field conditions (Table 1). An attempt was made to fit a simple Mendelian gene model to these data, but observed segregation ratios differed significantly from expectations for one and two (dominant or recessive) gene models as measured by chi-square goodness of fit tests. It is possible that environmental influences on expression of disease made it impossible to fit a simple gene model.

Data from the generation means analysis (10) (Table 1) for two crosses were used to test the additive-dominance genetic model. Ky 14 and Jaraiz 1 were highly susceptible, with a mean disease index of 4.0 in greenhouse experiments and 4.6 and 5.0, respectively, in the field experiment. HA 307 was highly resistant, with mean disease indexes of 1.0 for cross 1 and 1.4 for cross 2 in the greenhouse and 0.7 and 0.9, respectively, for each cross in the field. Differences in symptom expression are believed to be due primarily to temperature variations. The presence of mild virus symptoms in the resistant cultivar indicates that resistance does not involve an immune reaction. The virus replicated in this genotype but apparently caused no appreciable damage as judged by symptomatology. Field experiments reveal that HA 307 does not suffer significant yield reductions when inoculated shortly after transplanting (R. C. Rufty et al, *unpublished*). F_1 hybrids Ky 14 × HA 307 and Jaraiz 1 × HA 307 each showed mean disease indexes closely approximating midparent values. Mean disease indexes for F_2 generations were also similar in value to the midparent values. Mean disease indexes for backcross generations to the susceptible parents were approximately midway between those of the F_1 and the susceptible parents. A similar relationship was found for backcrosses of the F_1 hybrids to the resistant parent.

Results of Cavalli's (3) joint scaling test (chi-square goodness of fit test) indicate that the additive-dominance model is adequate in describing the major portion of variation among generations for TEV resistance with no evidence for epistasis (Table 2). The individual scaling tests (A, B, and C) (Table 2) did not deviate significantly from zero according to a *t* test and are thus in agreement with the results of the joint scaling test. Estimates for the genetic effects (*m*, [*d*], [*h*]) were obtained for both crosses from greenhouse and field experiments (Table 3). The major genetic effect was the additive effect with values between 1.4 and 1.8. The importance of additive gene effects is further substantiated by the fact that the F_1 means were approximately equal to midparent values. Estimates of dominance effects were not significantly different from zero according to a *t* test (10). This indicates a lack of dominance at individual loci or a lack of directional dominance across loci. The predominance of additivity in resistance to TEV should make incorporation of resistance into agronomically useful tobacco lines practical because genes for a susceptible or resistant disease reaction would not be masked by other dominant or epistatic alleles.

Frequency distributions of tobacco haploids in resistant and susceptible

Table 2. Joint scaling test (chi-square) and individual scaling tests (A, B, C) of the fit of the additive-dominance genetic model for the inheritance of resistance to tobacco etch virus in two crosses of burley tobacco under greenhouse and field conditions

Procedures		Cross 1 (Ky 14 × Havana 307)	Cross 2 (Jaraiz 1 × Havana 307)
Joint scaling test			
χ^2[a]	Greenhouse	0.1	0.1
	Field	0.9	1.2
Individual scaling tests			
A[b]	Greenhouse	0.1 ± 0.2 NS[c]	0.7 ± 1.0 NS
	Field	0.3 ± 0.4 NS	−0.6 ± 0.5 NS
B	Greenhouse	0.2 ± 0.3 NS	−0.1 ± 0.3 NS
	Field	0.5 ± 0.7 NS	−0.2 ± 0.3 NS
C	Greenhouse	−0.4 ± 0.4 NS	−0.4 ± 0.4 NS
	Field	0.7 ± 0.6 NS	0.5 ± 0.5 NS

[a] Calculated chi-square values compared with tabular value with 3 df obtained by equating observed family means to their expectations. Probability of a fit = 0.50–0.95, indicating adequacy of the additive-dominance genetic model.
[b] Where $A = 2\overline{BC}_1 - \overline{P}_1 - \overline{F}_1 = 0 \pm SE$, $B = 2\overline{BC}_2 - \overline{P}_2 - \overline{F}_1 = 0 \pm SE$, and $C = 4\overline{F}_2 - 2\overline{F}_1 - \overline{P}_1 - \overline{P}_2 = 0 \pm SE$.
[c] NS = Not significantly different from zero, indicating agreement with the joint scaling test (chi-square), i.e., adequacy of the additive-dominance genetic model.

Table 3. Estimates of genetic effects in two crosses of burley tobacco evaluated for resistance to tobacco etch virus under greenhouse and field conditions

Estimates of genetic effects[a]		Cross 1 (Ky 14 × Havana 307)	Cross 2 (Jaraiz × Havana 307)
m	Greenhouse	2.2	3.0
	Field	3.1	3.2
[*d*]	Greenhouse	1.5	1.4
	Field	1.8	1.7
[*h*]	Greenhouse	0.3	−0.4
	Field	0.3	0.4

[a] Where *m* = midparent value, [*d*] = the sum over loci of the additive effects, and [*h*] = the sum over loci of the dominance effects.

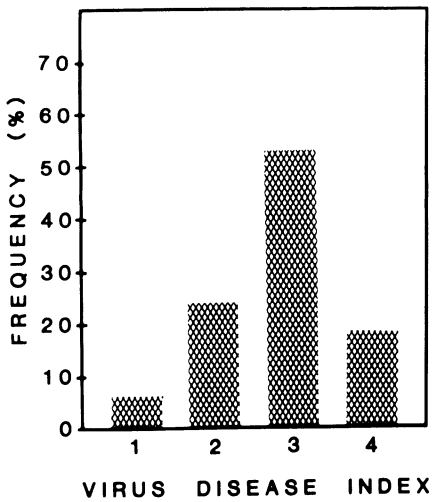

Fig. 2. Frequency distribution of resistant and susceptible tobacco haploids derived from F_1 hybrids Ky 14 × Havana 307 and Jaraiz 1 × Havana 307 inoculated with tobacco etch virus in a greenhouse. Virus disease index scale: 0 = no symptoms, 1 = < 1% leaf surface mottled, 2 = 1–5% mottled, 3 = 6–25% mottled, 4 = 26–50% mottled, 5 = > 50% mottled.

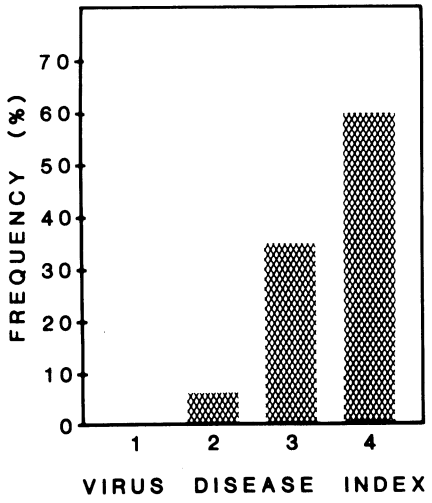

Fig. 3. Frequency distribution of resistant and susceptible tobacco doubled haploids derived from F_1 hybrid Jaraiz 1 × Havana 307 inoculated with tobacco etch virus in a greenhouse. Virus disease index scale: 0 = no symptoms, 1 = < 1% leaf surface mottled, 2 = 1–5% mottled, 3 = 6–25% mottled, 4 = 26–50% mottled, 5 = > 50% mottled.

classes are shown in Figure 2. Results are expressed as average frequencies among haploids derived from both F_1 hybrids, Ky 14 × HA 307 and Jaraiz 1 × HA 307. From a total of 1,164 haploids, 29.6% were classified as resistant (index 2). Frequency of highly resistant genotypes was lower in the haploid population than in the F_2 generation. Nevertheless, observed segregation ratios from TMV resistance vs. susceptibility and green vs. burley phenotypes did not significantly deviate from the expected ratios at the haploid level of 1:1 and 3:1, respectively. Because we did not see evidence of abnormal segregation in haploid genotypes for marker traits, we consider that segregation for TEV resistance was also normal.

Haploid plants derived from the F_1 hybrid Jaraiz 1 × HA 307 were picked at random for chromosome doubling in order to select for TEV resistance among DH lines in replicated tests and to compare the efficiency of selection at haploid vs. DH levels. Frequency distribution of DHs among the various resistant and susceptible classes is shown in Figure 3. None of the DHs were classified as highly resistant. Thus, frequency distribution among DHs differed from that of haploid genotypes in which several highly resistant genotypes were found. Furthermore, 60% of DH genotypes were classified as susceptible, compared with 18% of haploids. To state it differently, the haploid population approximated a normal distribution, whereas the DH population was skewed toward susceptibility. Explanations for this discrepancy include the fact that diploid plants are generally more vigorous and consequently may show more severe virus symptoms, or the differences may be simply due to sampling. There were 1,164 haploids and only 83 DHs; thus, it is possible that the relatively small sample of DHs did not contain highly resistant individuals. We do not interpret the results to indicate that selection is necessarily more efficient at the haploid level per se but rather that, all other things being equal, selection may be more effectively practiced among haploid genotypes than among DHs.

DH lines derived from haploids selected for TEV resistance in the greenhouse were evaluated in replicated trials under field conditions. The coefficient of correlation between virus reaction at the haploid level in the greenhouse and the reaction of DHs in the field was 0.73 ($n = 100$, significant at $P = 0.01$). This further demonstrates the efficacy of selection for TEV resistance at the haploid level in the greenhouse. Haploid selection for disease resistance has the advantage of reducing the time and labor required for chromosome doubling because only resistant genotypes need doubling as opposed to a priori chromosome doubling of all genotypes. Nevertheless, there is a possibility that some resistance may be lost in the process of haploidization and diploidization.

In conclusion, HA 307 appears to be a good source of resistance to TEV. On the basis of the high frequency of resistant genotypes in segregating generations and results of generation means analyses, we postulate a genetic model in which resistance is controlled by a few genes (possibly as few as two) with additive effects. Therefore, transfer of resistance should be a relatively easy task, barring unknown linkage relationships. The magnitude of the additive genetic effects relative to dominance effects indicates that resistance may be readily fixed in the inbreeding process normally used in tobacco to develop homozygous lines to be used as cultivars. Furthermore, the process of identification and selection of haploid resistant genotypes with subsequent chromosome doubling may be efficiently used to accelerate the development of TEV-resistant homozygous lines. HA 307 as a source of resistance to TVMV and potato virus Y is also being investigated.

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