such as those identified in the fragment amplified with the AB-nested primers have been reported to account for a substantial fraction of intraspecific cpDNA variation (Zurawski and Clegg 1987), presumably because such length changes can be tolerated in noncoding regions. When such sequence variation is assessed only by length comparisons as with the AB fragment, it is not possible to relate the observed length differences to specific mutational events. By sequencing 200 bp of the approximately 1000 bp AB fragment, the insertion/deletion events that resulted in the two length polymorphisms were defined. However, since seven length variants were identified for the entire AB fragment, complete sequence information for these seven fragments would likely provide useful information to further discriminate among the chloroplast types. If these seven AB length variants had been included in the haplotype analysis, 11 different haplotypes would have been identified.

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A Gene for Leaf Necrosis in Chickpea (*Cicer arietinum* L.)

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Necrosis of leaves was observed in the glabrous mutant (ICC 15566) of desi chickpea (*Cicer arietinum* L.). It was characterized by drying of leaflet margins to drying of complete leaflets of older leaves. The oldest leaves were the most affected and the intensity of necrosis decreased toward the apical meristem. A single recessive gene, designated *nec*, was found to govern the necrotic characteristic. The *nec* locus was linked to *gl* (glabrous shoots) with a map distance of 16 ± 3 cM. The loci *slv* (simple leaves), *mlv* (multipinnate leaves), *nlv* (narrow leaflets), *hg* (prostrate growth habit), *P* (pink corolla), and *shp* (round seed shape) segregated independently of *nec*.

A glabrous mutant of chickpea (*Cicer arietinum* L.) was identified by Pundir and Reddy (1989) in the M_2 of ethyl methane sulfonate (EMS) treated seeds of Chafa, a desi chickpea cultivar. The mutant was almost hairless in contrast to the dense hairs on normal chickpea and was highly susceptible to black aphid (*Aphis craccivora* Koch.). A single recessive gene, designated *gl*, was reported to govern the glabrous trait (Pundir and Reddy 1989).

In a study aimed at using the glabrous trait as a genetic marker in developing a linkage map of chickpea, we observed that the glabrous mutant had very poor vegetative growth and was highly infested by black aphids. The mutant also had necrotic leaves that progressed from lower (older) leaves to upper (younger) leaves. The appearance of similar necrosis on plants grown under chemical plant protection for insect pests ruled out the possibility of its occurrence due to biotic factors. Subsequent segregation analysis suggested that it was of genetic origin. This report identifies leaf necrosis as a new genetic trait in chickpea and describes its inheritance and linkage relationships with seven other morphological markers.

Materials and Methods

The glabrous mutant (ICC 15566) was crossed with five accessions of desi-type chickpea including ICC 5316 (multipinnate leaves, white flower, round seed shape), ICC 5434 (prostrate growth), ICC 10034 (narrow leaflets, double podded), ICC 10301 (simple leaf), and JG-315 (wilt resistance). The glabrous mutant was used as the male parent in all cross combinations because it was necrotic and weak. The F_1 and F_2 populations were grown under normal field conditions using recommended chemical control for insects. Observations were recorded on necrosis, glabrousness, and various other qualitative traits on individual F_2 plants. Inheritance and linkage analyses were performed using the computer program LINKAGE-1 (Suiter et al. 1983).

Results and Discussion

In necrotic plants, the necrosis began to appear prior to flower initiation. First the margins of the leaflets showed drying and then whole leaflets gradually dried. The intensity of necrosis was the highest on the oldest leaves and decreased gradually on the younger leaves. The apical meristem and a few younger leaves near it remained free from necrosis. The necrotic plants produced only a few pods.

All F_1 plants were normal, healthy, and vigorous. In the F_2, the frequencies of healthy and necrotic plants closely fit a 3:1 Mendelian ratio (Table 1), indicating that necrosis is governed by a single recessive nuclear gene. The gene symbol *nec* is proposed for this trait.

The linkage relations of *nec* were determined with several other morphological trait loci including *Gl/gl*, pubescent/glabrous shoot (Pundir and Reddy 1989); *Slv/slv*, normal pinnate/simple leaf (Ekbote 1942); *Mlv/mlv*, normal pinnate/multipinnate leaf (Pundir et al. 1990); *Nlv/nlv*, normal/narrow leaflets (Ramanujam and Singh 1945); *Hg/hg*, erect/prostrate plant growth habit (Argikar and D'Cruz 1963); *P/p*, pink/white corolla (Khan and Akhtar 1934); *Sfl/sfl*, single-podded/double-podded (D'Cruz and Tendulkar 1970); and *Shp/shp*, angular/round seed shape (Kazan et al. 1993). Our results confirmed the previously reported mode of inheritance at these loci. The contingency chi-square test indicated linkage between *nec* and *gl* in the F_2 of all five crosses (Table 2). An average distance of 15.8 ± 3.0 cM was estimated between these two loci. Healthy glabrous plants and necrotic pubescent plants were recovered as recombinants in the F_2, further ruling out the possibility of the occurrence of necrosis in glabrous plants due to their susceptibility to insect damage.

A study of joint segregation of the *nec* and *sfl* loci from the cross ICC 10034 × ICC 15566 indicated linkage between these loci (data not presented). This linkage, however, needs further confirmation as failure of expression of double poddedness in necrotic plants may be due to their poor vegetative growth. The loci *slv, mlv, nlv, hg, P,* and *shp* segregated independently of the *nec* locus (data not presented).

Table 2. Contingency χ^2 tests for joint segregation of *nec* (necrosis) and *gl* (glabrous) loci in F_2 of chickpea

F_2 population	F_2 phenotype[a] PU/HL	GL/HL	PU/NC	GL/NC	χ^2	P	r ± SE
ICC 5316 × ICC 15566	157	13	12	30	84.7	<0.001	13.8 ± 6.7
ICC 5434 × ICC 15566	138	20	14	36	68.0	<0.001	17.8 ± 6.7
ICC 10034 × ICC 15566	145	9	11	24	77.9	<0.001	12.7 ± 7.1
ICC 10301 × 1CC 15566	188	19	25	41	81.8	<0.001	18.4 ± 5.8
JG 315 × 1CC 15566	81	8	11	28	52.9	<0.001	15.0 ± 8.6
Pooled data					364.0	<0.001	15.8 ± 3.0
Heterogeneity					1.3	0.860	

[a] PU = pubescent, GL = glabrous, HL = healthy, NC = necrotic.

The glabrous mutant of chickpea may prove useful in entomological and pathological studies because of its high susceptibility to insects (Pundir and Reddy 1989). For example, the glabrous mutant would be a good medium for rearing large populations of black aphids required for studies of stunt disease transmission. We found that the usefulness of the glabrous mutant was adversely affected by its heritable necrosis. Necrosis reduces the photosynthetic area of the plant by partial or complete drying of leaves and leads to poor vegetative growth. We have recovered healthy glabrous recombinant plants that will be more useful in this respect than the original necrotic glabrous mutant. Moreover, the glabrous trait is now available in the background of different leaf types, namely, simple leaves, multipinnate leaves, and narrow leaflets.

Even though necrosis is an agronomically undesirable character, it is a valuable genetic marker for gene mapping. The linkage between *nec* and *gl* loci is a good addition to the information on genetic linkage in chickpea. Attempts are being made using isozyme markers to assign this linkage to the previously reported isozyme linkage groups of chickpea (Gaur and Slinkard 1990a,b; Kazan et al. 1993).

The genera *Cicer* and *Pisum* are closely related and have several common linkage groups consisting of homologous genes (Gaur and Slinkard 1990a,b; Kazan et al. 1993; Simon and Muehlbauer 1997). A mutant characterized by brownish, papery, necrotic margins of the leaflets and stipules, designated "burnt leaf," has been reported in *Pisum* (Sharma 1973). Initially only the tips of leaflets and stipules show a burnt appearance which subsequently spreads to whole leaflets and stipules. A recessive gene, designated *bulf*, was found to control this mutation. The *bulf* gene of *Pisum* does not appear homologous to the *nec* gene of *Cicer* as the *bulf* gene affects the growing tips more severely than the lower part of the plant, whereas the opposite is the case with the *nec* gene of *Cicer*. The *bulf* gene has been mapped on chromosome 3 of *Pisum* close to *st* for reduced stipules and *chi-6* for light green to yellowish green plant (Marx 1980). The location of the *gl-nec* linkage on the gene map of *Cicer* is yet to be determined.

Table 1. Goodness-of-fit χ^2 tests for 3:1 single-locus F_2 segregation for necrosis and glabrous loci in chickpea

	F_2 phenotype		Goodness-of-fit		Heterogeneity	
Trait/F_2 population	Dominant	Recessive	χ^2	P	χ^2	P
Necrosis	Normal	Necrotic				
ICC 5316 × ICC 15566	170	42	3.04	0.08		
ICC 5434 × ICC 15566	158	50	0.10	0.75		
ICC 10034 × ICC 15566	154	35	4.23	0.04		
ICC 10301 × 1CC 15566	207	66	0.10	0.75		
JG 315 × 1CC 15566	89	39	2.04	0.15		
Pooled data			2.22	0.14	7.30	0.12
Glabrousness	Pubescent	Glabrous				
ICC 5316 × ICC 15566	169	43	2.51	0.11		
ICC 5434 × ICC 15566	152	56	0.41	0.52		
ICC 10034 × ICC 15566	156	33	5.73	0.02		
ICC 10301 × 1CC 15566	213	60	1.33	0.25		
JG 315 × 1CC 15566	92	36	0.67	0.41		
Pooled data			3.17	0.08	7.48	0.11

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Single-Locus Inheritance in the Allotetraploid *Coffea arabica* L. and Interspecific Hybrid *C. arabica* × *C. canephora*

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Molecular cytogenetic analysis has indicated that *Coffea arabica* is an amphidiploid formed from the hybridization between two closely related diploid progenitor species, *C. canephora* and *C. eugenioides.* Our aim was to determine the mode of inheritance in *C. arabica* and in a tetraploid interspecific hybrid (called arabusta) between *C. arabica* and *C. canephora* as revealed by segregation analyses of restriction fragment length polymorphism (RFLP) loci markers. The observed RFLP allele segregations in an F_2 progeny of *C. arabica* conform to disomic inheritance as expected, with regular bivalent pairing of homologous chromosomes in the F_1 hybrid. In contrast, RFLP loci followed tetrasomic inheritance in the arabusta interspecific hybrid, although bivalents have been reported to predominate greatly at meiosis in its hybrid. These results suggest that homologous chromosomes do not pair in *C. arabica,* not as a consequence of structural differentiation, but because of the functioning of pairing regulating factors. Moreover, the arabusta hybrid seems to offer the possibility of gene exchange between the homologous genomes.

Table 1. Meiotic configurations reported in *Coffea arabica*, in tetraploid *C. canephora* and in interspecific arabusta hybrids (*C. arabica* × *C. canephora* 4*x*)

		Frequency of chromosome association per pollen mother cell at metaphase I[a]			
Species/accessions	References	I	II	III	IV
C. arabica cv. SL28	Owuor (1985)	1.8	19.9	0.1	0.5
C. arabica cv Caturra,	Grassias and Kammacher (1975)	0.5	21.5	0.0	0.1
Tetraploid *C. canephora*	Grassias (1980)	1.6 ± 0.4	14.0 ± 1.0	0.7 ± 0.2	3.0 ± 0.6
Tetraploid *C. canephora*	Boaventura (1990)	3.6	15.2	0.7	1.9
Tetraploid *C. canephora*	Owuor (1985)	2.4 ± 1.8	14.9 ± 0.9	0.8 ± 1.7	2.3 ± 1.0
Arabusta hybrids	Owuor (1985)	6.1 ± 0.8	17.4 ± 0.4	0.8 ± 0.1	0.2 ± 0.2
Arabusta hybrids	Grassias (1980)	5.8–8.5	13.4–15.9	1.3–2.5	0.3–1.0

[a] I = univalent; II = bivalent; III = trivalent; IV = tetravalent.

In spite of its economic importance, genetic research devoted to *Coffea arabica* ($2n = 4x = 44$), the only polyploid species in the genus, has been rather limited. According to recent molecular cytogenetic investigations (Lashermes et al. 1999), *C. arabica* is an amphidiploid formed from the hybridization between two closely related diploid species ($2n = 22$), *C. canephora* and *C. eugenioides.* The evidence suggests recent speciation and low divergence between the two constitutive genomes of *C. arabica* and those of its progenitor species. In spite of the close relationship between the two constitutive genomes, *C. arabica* is considered to display diploidlike meiotic behavior (Krug and Mendes 1940; Table 1).

Polyploidy is a major process of genome evolution that can promote rapid speciation (deWet 1980). In particular, allopolyploidy arising as a result of combining related, but not completely homologous, genomes is very common among angiosperms. The stabilization of allopolyploids requires a restriction of pairing and genetic recombination between the different parental chromosomes related by ancestral homology (Moore 1998). Thus allopolyploids are characterized by bivalent formation during meiosis and disomic inheritance (Burnham 1962; Stebbins 1950). On the other hand, the ability to transfer useful traits from a relative species to the cultivated form by conventional methods depends on the genomic affinity between the two species (Kimber 1984). For instance, little or no exchange between the homologous genomes would be expected if there is no interspecies chromosome pairing.

Analysis of the mode of inheritance can provide an indication of the degree of intergenome chromosome pairing and therefore an assessment of recombination potential between the different genomes. The mode of inheritance can be deduced from examinations of segregation of alleles at a number of individual loci. Until recently, analyses of this type in coffee trees have not been feasible because of a lack of suitable genetic markers. The development of restriction fragment length polymorphism (RFLP) markers representing various loci with multiple codominant alleles means that such analysis can now be envisaged.

In the our study, RFLP markers were used to determine the mode of inheritance in *C. arabica* and in a tetraploid interspecific hybrid called arabusta (Capot 1972) between *C. arabica* and an autotetraploid form of one of its diploid progenitors, *C. canephora.* Results are discussed in relation to the chromosome meiotic behavior and the possibility of gene exchange between the homologous genomes in *C. arabica,* as well as in interspecific hybrids between *C. arabica* and diploid relatives.

Materials and Methods

The segregating material surveyed consisted of 14 F_2 plants of *C. arabica* obtained by controlled selfing of the F_1 hybrid (Et 30 × Caturra), and 70 plants resulting from the backcross of a tetraploid interspecific arabusta F_1 hybrid (Et 30 × IF 181T) to *C. arabica* (accession Et 30). The arabusta hybrid resulted from a cross between a plant of *C. arabica* (accession Et 30) used as the female parent, and a tetraploid plant of *C. canephora,* IF