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The mapping of phytochrome genes and photomorphogenic mutants of tomato

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Abstract The map positions of five previously described phytochrome genes have been determined in tomato (*Lycopersicon esculentum* Mill.) The position of the *yg-2* gene on chromosome 12 has been confirmed and the classical map revised. The position of the phytochrome A (phy A)-deficient *fri* mutants has been refined by revising the classical map of chromosome 10. The position of the *PhyA* gene is indistinguishable from that of the *fri* locus. The putative phyB1-deficient *tri* mutants were mapped by classical and RFLP analysis to chromosome 1. The *PhyB1* gene, as predicted, was located at the same position. Several mutants with the *high pigment* (*hp*) phenotype, which exaggerates phytochrome responses, have been reported. Allelism tests confirmed that the *hp-2* mutant is not allelic to other previously described *hp* (proposed here to be called *hp-1*) mutants and a second stronger *hp-2* allele (*hp-2^j*) was identified. The *hp-2* gene was mapped to the classical, as well as the RFLP, map of chromosome 1.

Key words Phytochrome · Tomato · RFLPs · Photomorphogenic mutants · *high pigment* mutants

Introduction

Light plays a crucial role in the life cycle of plants. To sense light quantity and quality plants possess different types of photoreceptors, of which the red/far-red light-absorbing phytochrome is the best characterized. The existence of a number of phytochrome proteins encoded by different genes (Quail 1994; Pratt 1995), which in tomato can have specific expression patterns (Hauser et al. 1995), adds to the complexity of phytochrome signal transduction. It is assumed that the different phytochrome proteins share the same linear tetrapyrrole chromophore (Terry et al. 1993). Whether the different phytochromes use the same, or different, signal transduction pathways is not yet clear.

The genetic basis of photomorphogenesis can be investigated by using mutants specific either for the photoreceptor itself or for the transduction chain. Such mutants can help in attributing physiological roles to specific components of the system. Mutants in the phytochrome A and B protein and chromophore mutants, which are deficient in all types of phytochrome, have been described for several species (Koornneef and Kendrick 1994). Two different classes of signal transduction mutants have been identified: mutants that apparently mimic the light signal, such as the *det* and *cop* mutants in *Arabidopsis*, and mutants resembling the photoreceptor mutants in overall phenotype, such as *hy5* in *Arabidopsis* (reviewed by McNellis and Deng 1995). By molecular characterization of mutants at the protein, mRNA, and DNA level it is possible to link specific mutants with cloned genes. The localization of a mutant and a specific gene at similar positions on the genome is often an important indication that the mutation affects that particular gene. When sequence information of a mutant allele is not available, the map position of a locus defined by a mutant phenotype can be a starting point for cloning that particular locus (Tanksley et al. 1995). A problem, when comparing map positions of mutants on the classical map with positions of molecular markers on the molecular map, is that often different mapping populations and reference markers have been used for the construction of the two types of map. A further complication, e.g. in a species such as tomato, is that the classical maps (Tanksley et al. 1992) are often inaccurate. To solve the problem of the inte-

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gration of classical and molecular maps, morphological markers should be mapped in relation to molecular markers and, in addition, the quality of the classical marker map needs to be improved. The latter can be achieved by adding new data and using software that is able to integrate linkage data. In tomato (*Lycopersicon esculentum* Mill.) this integration of classical and molecuar maps has already been achieved for the top part of chromosome 1 (Balint-Kurti et al. 1995), for chromosome 3 (Koornneef et al. 1993; Van der Biezen et al. 1994), and for chromosome 6 (Weide et al. 1993; Van Wordragen et al. 1996). In addition, miscellaneous markers have been integrated in the standard molecular map (Tanksley et al. 1992).

In tomato, the phytochrome gene family appears to consist of as many as 9–13 members (Hauser et al. 1995) of which five (designated *PhyA*, *PhyB1*, *PhyB2*, *PhyE* and *PhyF*) have been characterized more throughly (Hauser et al. 1995; Pratt 1995). Their position on the linkage map, however, is still unknown. This is also true of some of the recently discovered and even of the well-known photomorphogenic mutants in tomato.

Sharrock et al. (1988), using a RFLP for a phytochrome coding sequence from *Arabidopsis*, which they presumed was *PhyA*, mapped this locus to tomato chromosome 10. Van Tuinen et al. (1995a) described the isolation and characterization of two allelic phyA-deficient mutants (*fri*^{1} and *fri*^{2}) in tomato and reported linkage with chromosome-10 morphological markers. The fact that the phyA-deficient mutants mapped to chromosome 10 suggests that Sharrock et al. (1988) indeed mapped the *PhyA* homologous gene.

The *tri* mutant of tomato, unlike the previously described phyB-deficient mutants of *Arabidopsis* (Reed et al. 1993), cucumber (López-Juez et al. 1992) and *Brassica rapa* (Devlin et al. 1992), still exhibits a strong end-of-day far-red response (Van Tuinen et al. 1995b). Tomato, like *Arabidopsis*, has two type-B phytochromes. However, analysis revealed that this has arisen as a result of independent duplications of a *PhyB* progenitor gene in the *Solanaceae* and *Brassicaceae* (Pratt et al. 1995). Molecular analysis of the mRNAs of the *tri*-mutant alleles points to the *Tri* locus being the gene encoding the apoprotein of phytochrome B1 (Kerckhoffs et al. 1996). Localization at the same position on the chromosome would give further evidence for the view that the *tri* mutants are defective in the *PhyB1* gene itself.

Chromophore mutants in tomato are represented by the *au* and *yg-2* mutations (Terry and Kendrick 1996; Van Tuinen et al. 1996). The position of the *au* mutant is firmly established on the classical, as well as the RFLP, map of chromosome 1 (Balint-Kurti et al. 1995). For *yg-2*, however, the suggestion that it might be on chromosome 12 (Kerr 1979c) has never been confirmed.

A type of transduction-chain mutant, which thus far has only been described in tomato, is the *high pigment* (*hp*) mutant (Peters et al. 1989) which shows an exaggerated phytochrome response, and no difference in phytochrome content compared to the wild-type has yet been found (Peters et al. 1992). The first *hp* gene was reported as early as 1917 (Reynard 1956). Soressi (1975) described a mutant that resembled *hp* phenotypically, but found that it was non-allelic to *hp* and designated it *hp-2*. Mochizuki and Kamimura (1986), however, observed an *hp* phenotype in the F_1 and a lack of segregation in F_2 populations derived from crosses between *hp* and *hp-2* and concluded that *hp-2* was allelic to *hp*.

The present report describes the mapping of five different phytochrome genes in tomato on the basis of their RFLP pattern using gene-specific probes. In addition, we mapped the recently isolated *fri* and *tri* mutants. The map positions confirmed their relationship with the *PhyA* gene on chromosome 10 and *PhyB1* gene on chromosome 1, respectively. This allowed a better integration of the classical and molecular maps of chromosomes 1 and 10. A revised classical map was made for chromosome 10 by adding recent linkage data to all data on chromosome 10 available in the literature. Additional linkage data were obtained for *au* and *yg-2*, whose location on chromosome 12 was confirmed. The conflict in the literature on the existance of a distinct *hp-2* locus was resolved and the gene was mapped on chromosome 1, using both morphological and RFLP markers.

Materials and methods

Plant materials

Mutants

The *fri* and *tri* mutants used have been described by Van Tuinen et al. (1995a, b), and the *au* and *yg-2* mutants by Koornneef et al. (1985), Van Tuinen et al. (1996) and references therein. A mutant with dark-green immature fruits, similar to the extreme *hp* allele, *hp*^{w} (WB3) (Peters et al. 1989), was obtained from Drs. David and Jonathan Jones (Sainsbury laboratory, Norwich, UK), who found this mutant in the progeny of a T-DNA-transformed plant (cv Moneymaker). This mutant phenotype did not co-segregate with the T-DNA. Allelism tests revealed that the mutant is allelic to the *hp-2* mutant (Soressi 1975) received from Dr. Soressi. We propose the gene symbol *hp-2*^{j} for this new *hp-2* allele.

Genotypes homozygous for several morphological markers were obtained from the Tomato Genetic Resource Center (Davis, Calif., USA) or have been constructed in Wageningen (Koornneef et al. 1990).

Mapping populations

For the molecular mapping of the *tri* and *hp-2* mutants several backcross (BC) populations have been used. A *L. esculentum* line carrying the *tri*^{1} mutant allele was crossed with *L. pennellii* (LA716). An F_1 plant was used as a male parent in a backcross to the *esculentum* line carrying the *tri*^{1} mutant allele. This generated a BC_1 population of 28 plants. For *hp-2* the procedure outlined above was followed. In the *pennelli* background, however, it was impossible to score the *hp* phenotype (Peters et al. 1989) unambiguously, since both *hp* seedling phenotype and fruit colour were modified by *L. pennellii* genes and intermediate phenotypes were found. From this BC_1 population DNA was isolated from plants with clear phenotypes only, generating a population of 20 individuals. Two BC_1 plants with clear wild-type fruit colour were selected and backcrossed again, this time to a line carrying the more extreme *hp-2*^{j} allele described above. This generated two BC_2 populations of 42 and 49 individuals, respectively, which segregated in a ratio of 1:1 for wild-type to *hp* fruit colour, indicating the absence of modifying loci. The *tri* and *hp-2* phenotypes were scored at the seedling level after a 7-day period of continuous red light (Philips TL40/103339 filtered through one layer of primary red filter (Lee, Flashlight Sales B.V., Utrecht, The Netherlands; 3 μmol m^{-2} s^{-1}). The *hp* phenotype was confirmed at the adult plant stage by fruit colour.

To locate the various phytochrome genes, an F_2 mapping population (84 plants), derived from a cross between *L. esculentum* cv Allround and *L. pennellii* (LA716) (Odinot et al. 1992), was used. DNA of these plants was kindly provided by Dr. P. Lindhout [Centre for Plant Breeding and Reproduction Research (CPRO-DLO), Wageningen, The Netherlands].

For the mapping of *yg-2*, the *yg-2* mutant was crossed with a line (LA1177) carrying the chromosome-12 markers *alb* (Khush and Rick 1966) and *mua* (Zobel et al. 1969). Linkage analysis performed in the F_2 population produced one *yg-2*, *mua* recombinant, which did not segregate *alb* in the F_3. To obtain more linkage data the *yg-2*, *mua* line was crossed to lines carrying the *fd* mutation (Rick et al. 1967; LA873) or the *alb* and *fd* mutations (LA1111).

DNA methodology

Plant DNA was isolated from leaves as described by Van der Beek et al. (1992), digested with the appropriate restriction enzymes, separated by electrophoresis on 1% (w/v) agarose gels, denatured and blotted onto Genescreen Plus (NEN Research Products, Boston, Mass.) hybridization membranes. Probes were labelled with ^{32}P-dATP using the "random primer" method according to Feinberg and Vogelstein (1983). Hybridization of Southern blots was performed as stated by Klein-Lankhorst et al. (1991).

Probes and hybridization conditions

For the molecular mapping of the phytochrome genes, PHY fragments ($PHYA_{212/320}$, $PHYB1_{212/430}$, $PHYB2_{212/430}$, $PHYE_{212/320}$ and $PHYF_{212/320}$; Hauser et al. 1995) were cut out of their bluescript vector and separated from vector DNA on a 1% agarose gel. The tomato DNA was isolated from the agarose using the GENECLEAN (Bio 101 Inc., La Jolla, Calif.) procedure according to the manufacturer's instructions. The cleaned DNA was then used for the preparation of radioactive labelled probes.

The phytochrome genes were mapped using tomato genomic (TG) clones, with known positions, developed by Tanksley et al. (1992). After PCR of the inserts using M13 primers the PCR product was used as a probe either directly or after cleaning by the freeze-squeeze method (Ogden and Adams 1987).

Linkage analysis and map construction

In order to identify RFLPs suitable for mapping the phytochrome genes, DNA from *L. esculentum* cv Moneymaker and *L. pennellii* (LA716) was digested with 14 restriction enzymes. Southern-blot analysis, using the radioactively labelled PHYA, PHYB1, PHYB2, PHYE and PHYF probes, revealed polymorphisms for *Taq*I, *Hin*dIII, *Hin*dIII, *Hin*cII and *Hae*III, respectively. Subsequently, the probes were hybridized to Southern blots containing DNA (digested with the appropriate restriction enzyme) of the F_2 population described above (plant material). This F_2 population, characterized with several RFLP markers common to the map constructed by Tanksley et al. (1992), has been used previously for mapping studies (Liharska et al. 1994; Arens et al. 1995; Bonnema et al. 1996). We added our data on the phytochrome genes and screened the population with additional RFLP markers. These were TG51, TG125 for chromosome 1; TG14, TG204, TG312, TG554 for chromosome 2; CD78, CT80, TG623 for chromosome 5; TG183, TG202 for chromosome 7; and TG303, TG566, TG596 for chromosome 10 (Tanksley et al. 1992). The BC populations were scored for the segregation of either *tri* or *hp-2* and the RFLP segregation for PHYB1, LYC52 (Bonnema et al. 1996) and the chromosome-1 markers TG24, TG51, TG125, TG378 and CT151 (Tanksley et al. 1992).

Segregation data were analyzed with the computer programme JOINMAP (Stam 1993). Graphical depiction of the maps was accomplished by the programme DRAWMAP (Van Ooijen 1994).

Results and discussion

Mapping of the *tri* and *hp-2* loci, and the *PhyB1* gene on chromosome 1

Mochizuki and Kamimura (1986) stated that *hp-2* (Soressi 1975) was allelic to *hp*. Based on our allelism tests between *hp-2^j* and different *hp* mutants. (A. van Tuinen, unpublished data), we concluded that *hp-2* is not allelic to *hp*. Moreover, the tests revealed a second *hp-2* allele, *hp-2^j*. We therefore propose to change the gene symbol *hp* into *hp-1*.

Since it was difficult to find the *au*, *hp-2* and *au*, *tri* double mutants we suspected that both *hp-2* and *tri* were located on chromosome 1. The *hp-2* and *tri* mutants were therefore crossed with lines carrying chromosome-1 morphological markers and linkage was detected (Table 1). Our data (Table 1), combined with those used by Balint-Kurti et al. (1995), were used to construct a revised classical map of the top part of chromosome 1 (Fig. 1). For the construction of the RFLP map, individual plants of four BC populations were scored for *tri*, *hp-2* or the wild-type phenotype and used for RFLP analysis. The BC data were combined with data from the

Table 1 New linkage data on classical markers on chromosomes 1

Markers	Phase[a]	Genotype classes				Total	χ^2_{ass}	Rec.[b]
		A.B.	*aaB.*	*A.bb*	*aabb*			
au-hp-2	C	342	34	26	76	478	193.8*	14.1 ± 1.7
au-tri	C	259	21	26	62	368	152.4*	14.1 ± 2.0
au-gib-2	R	254	98	116	12	480	18.1*	32.0 ± 4.0
au-scf	C	233	35	68	13	349	0.5	45.7 ± 3.8
hp-2-gib-2	R	256	96	120	6	478	28.0*	23.9 ± 4.3
hp-2-scf	R	136	38	32	6	212	0.7	46.7 ± 5.3
tri-scf	R	102	40	19	4	165	1.2	41.2 ± 6.4

* Significant at $P < 0.001$

[a] C: coupling phase; R: repulsion phase

[b] Rec. is recombinant percentage with SE calculated with RECF2

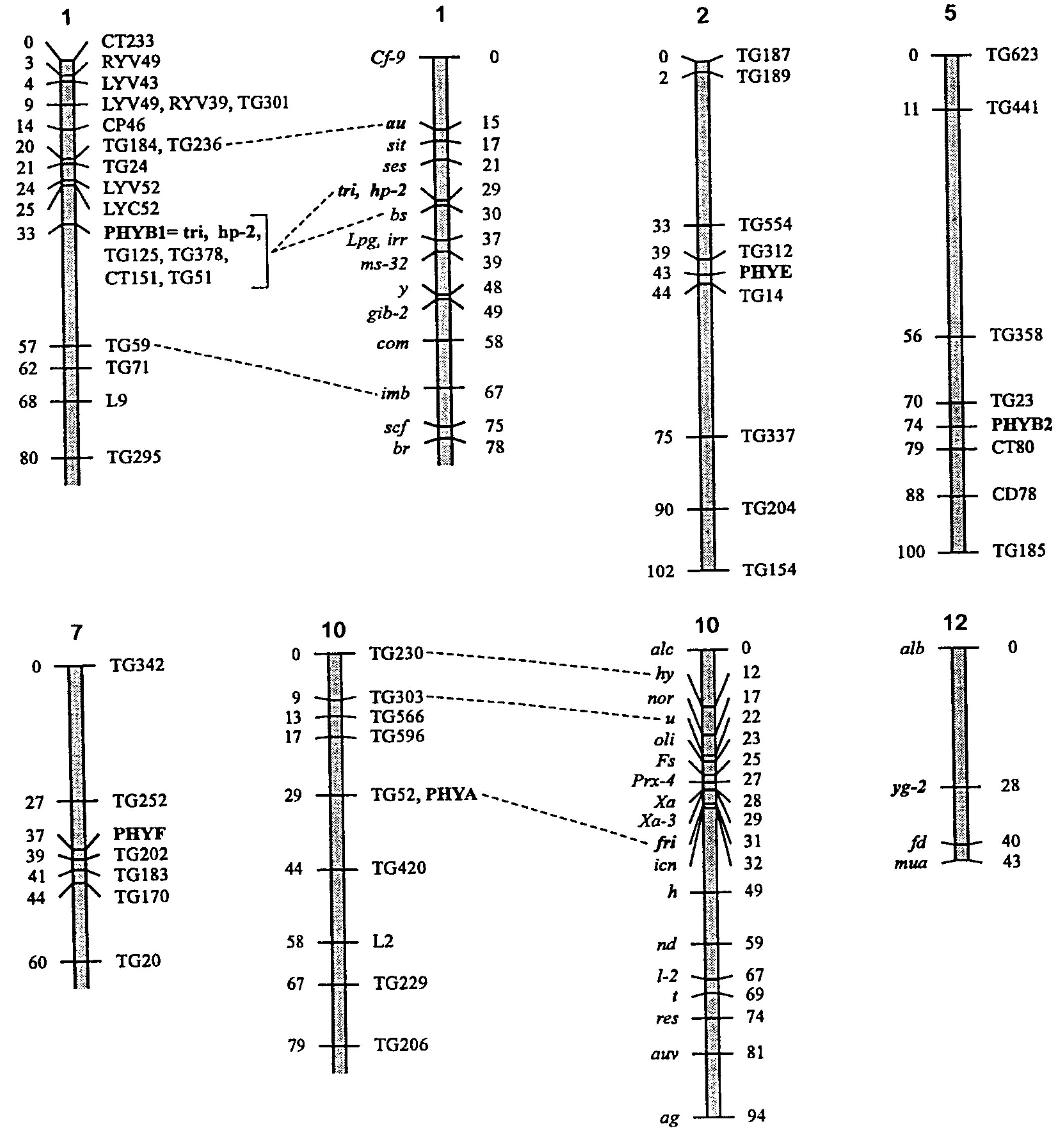

Fig. 1 RFLP (markers to right) and classical (markers to left) maps of tomato chromosomes showing the map positions of the phytochrome genes and photomorphogenic mutants. Recombination distances for the RFLP maps of chromosomes 2, 5, 7 and 10 were based upon the analysis of 84 *L. esculentum* × *L. pennellii* F_2 plants (Odinot et al. 1992). For the RFLP map of chromosome 1 the F_2 data were combined with data on four *L. esculentum* × *L. pennellii* BC populations (see Materials and methods). The map orientations are according to maps published by Balint-Kurti et al. (1995; chromosome-1 classical map), Bonnema et al. (1996; chromosome-1 RFLP map), Frary et al. (1996; chromosome 7) and Tanksley et al. (1992; chromosomes 2, 5, 10 and 12). *Dotted lines* represent the integration between the RFLP and classical maps of chromosomes 1 (Balint-Kurti et al. 1995; this article) and 10 (Tanksley et al. 1992; this article)

F_2 population using JOINMAP (Stam 1993) to construct the RFLP map (Fig. 1). The absence of recombinants between *tri* and *PHYB1* is consistent with the observation that mRNA analysis points to the *Tri* locus being the gene encoding the apoprotein of phytochrome B1 (Kerckhoffs et al. 1996). The *tri* and *hp-2* genes map to the same position on the chromosome in the RFLP map. This coincides with their similar position on the updated classical map (Fig. 1).

A revised classical map of chromosome 10 and molecular mapping of the *PhyA* gene

We reported previously that the phyA-deficient *fri* mutants mapped to chromosome 10 (Van Tuinen et al. 1995a). The map position of *fri* was then calculated relative to the map position of *u* on the linkage map published by Tanksley et al. (1992). Linkage data presented for the *fri* mutants (Van Tuinen et al. 1995a) and those from the literature (McArthur 1934; Butler 1955, 1956; Rick 1955, 1956; Butler and Chang 1958; Burdick 1958; Kerr 1958, 1960, 1966, 1967, 1979a, 1982a, b, c; Chiscon 1960; Clayberg 1962; Hansen et al. 1962; Reeves et al. 1967; Rick et al. 1968, 1974; Tigchelaar et al. 1973; Rick and Fobes 1977; Tigchelaar and Barman 1978; Mutschler 1984) that could be verified [e.g. re-calculated with the computer programme RECF2 (Koornneef and Stam 1992)] were combined to construct the revised classical map of chromosome 10 (Fig. 1), using the computer programme JOINMAP (Stam 1993). The *ten* locus could no longer be placed on the map because only reliable linkage data with one marker (Reeves et al. 1966) were available. For *sh* the indication of linkage to chromosome 10 (Lesley and Lesley 1971, 1980) was later confirmed by Kerr (1982c). The data on linkage for *sh* with *h* and *t* obtained by Kerr (1982c) could not be used, due to the lethality of the *XaXa* plants, leaving only an estimation of linkage with *Xa*. Although Andrásfalvy (1968) reported linkage with *h* for *ms-31*, the results of the trisomic analysis by Reeves (1972) were inconclusive. No further data for *ms-31* have been found; its position on chromosome 10 is therefore only tentative. Rick et al. (1994) reported *Abg*, a gene for purple fruit derived from *Solanum lycopersicoides*, which co-segregated with the chromosome-10 RAPD marker Do3-1200. Further data are necessary to verify its position on chromosome 10. Semi-dominant chlorophyll mutants with seedling lethality of the homozygous dominant have been found in tomato and named *Xanthophyllic* (*Xa*). Three different *Xa* loci have been described: *Xa* (Young and McArthur 1947), *Xa-2* (Persson 1960; Anonymous 1963) and *Xa-3* (Gröber 1963). However, no reports of allelism tests were found in the literature. Due to the absence of linkage data that could be verified, *Xa-2* could not be placed on the new classical map (Fig. 1). The fact that *Xa* and *Xa-3* map to approximately the same position on the new classical map might indicate allelism. The position of *oli* on the map published by Tanksley et al. (1992) is based on Kerr's (1977) linkage summary. However, Kerr did not include his earlier linkage data with *u* and *h* (Kerr 1967) or the linkage data with *h* presented by Hansen et al. (1962). The movement of *oli* from the long arm to the short arm can therefore be explained by the fact that it is unlinked to *ag* (Kerr 1967), coupled with its relatively loose linkage with *t* and *l-2* (Kerr 1977) and the 'forgotten' stronger linkage data with *h* (Hansen et al. 1962; Kerr 1967).

The *PhyA* gene was mapped by RFLP analysis to the TG52 cluster on chromosome 10 (Fig. 1). The fact that the *fri* gene maps to a position on the classical map comparable to that of the *PhyA* gene on the RFLP map supports the hypothesis that the *fri* mutants are defective in the *PhyA* gene itself. Preliminary results indicate that the DNA sequence of both *fri* alleles differs from the wild-type DNA sequence (G. Lazarova et al. 1996).

Molecular mapping of the *PhyB2*, *PhyE* and *PhyF* genes

The DNA sequence-homology of the *PhyB2* gene with the *PhyB1* gene is 84% for the 212–430 region (Pratt et al. 1995). The *PhyB2* gene does not map in the vicinity of the *PhyB1* gene on chromosome 1, but maps to chromosome 5 (Fig. 1). The *PhyE* and *PhyF* genes were located on chromosomes 2 and 7, respectively (Fig. 1).

Mapping of the *yg-2* gene

The classical linkage map of chromosome 12 is poorly developed and contains only the markers *alb*, *fd* and *mua*, the position of the latter being even indicated with a question mark (Tanksley et al. 1992). The reliability of the *Cf-11* position has also been questioned (P. Lindhout personal communication). Linkage of *yg-2* with *alb* and *fd* has been found (Rick et al. 1968; Kerr 1979), but the locus was not placed on the map (Tanksley et al. 1992).

Linkage analysis of F_2 populations derived from crosses of the *yg-2* mutant with tester lines homozygous for several morphological markers of chromosome 12 revealed significant linkages with all markers used, thus confirming the observation of Kerr (1979c). Therefore all linkage data on chromosome 12 were collected and checked with the computer programme RECF2 (Koornneef and Stam 1992). After this check the literature data were pooled with our data and the recombination percentages given in Table 2 obtained. The revised classical map constructed with these data is given in Fig. 1.

Based upon linkage data for *hp-1* and *yg-2* from two small F_2 populations in repulsion phase and very weak linkage of *hp-1* with *alb* and *fd*, Kerr (1979b) concluded that *hp-1* also resided on chromosome 12. However, we found no significant linkage between *hp-1* and the chromosome-12 markers *alb* and *mua*.

Table 2 Data on linkage between classical markers on chromosome 12

Markers	Phase[a]	Genotype classes				Total	χ^2_{ass}	Rec.[c]
		A.B.	*aaB.*	*A.bb*	*aabb*			
alb–yg-2	R	462	219	191	8	880[d]	63.6	20.0 ± 3.2
alb–fd	C	144	25	18	14	201	14.4	29.4 ± 3.9
alb–fd	R	1186	410	263	24	1883[e]	41.2	34.3 ± 2.0
alb–mua	C	895	225	197	121	1438	43.7	37.8 ± 1.7
alb–mua	R	310	100	113	16	539[f]	8.4	38.8 ± 3.6
yg-2–fd	R	336	188	82	0	606[g]	42.6	0.0 ± 4.1
yg-2–mua	C	248	24	22	93	387	207.6	13.8 ± 2.2
yg-2–mua	R	108	52	71	1	232	27.2	11.9 ± 6.5
fd–mua	R	384	108	236	0	728[f]	60.8	0.0 ± 3.7

[a] C: coupling phase; R: repulsion phase

[b] All data significant at the 0.01 probability level

[c] Rec. is recombination percentage ± SE calculated with RECF2

[d] Data from Rick et al. (1968) and Kerr (1979) are included

[e] Literature data only (Rick et al. 1968)

[f] Data with recombinant percentage < 50 from Zobel et al. (1969) are included

[g] Data from Rick et al. (1968) are included

Moreover, when *hp-1* plants, from an F_2 derived from a cross between *hp-1* and *yg-2*, were selected and allowed to self, 23 of the 30 F_3 lines obtained segregated *yg-2* plants. This does not significantly deviate from the expected 2:1 ($\chi^2 = 1.35$; $P > 0.05$) ratio of segregating to non-segregating lines, giving no indication for linkage between *hp-1* and *yg-2* and indicating that *hp-1* is not on chromosome 12.

Concluding remarks

Several phytochrome genes have been mapped in different species (Hauge et al. 1993; Paterson et al. 1995). In tomato, the phytochrome genes mapped so far are not clustered, but distributed over different chromosomes of the genome. This is in contrast to the cereals sorghum and rice, where the *PhyA*, *PhyB* and *PhyC* genes map to the same chromosome (A. Paterson, L. H. Pratt and M.-M. Cordonnier-Pratt personal communication), but is comparable to the situation in *Arabidopsis* (Hauge et al. 1993).

The *au* and *yg-2* genes are most likely orthologues of the *Arabidopsis hy1* and *hy2* genes (Parks and Quail 1991). If cloning in *Arabidopsis* is successful, the *Arabidopsis* probes might be used in tomato to confirm co-localization.

The mapping of the *hp-2* and *tri* genes to the same cluster on chromosome 1 does not necessarily mean that the genes are related at the DNA level. The localization of *hp-2* and *tri* in a large cluster of markers might indicate suppression of recombination, which implies that the physical distances between the various markers might be considerable. In tomato such clusters of loci appear to be located near the centromeres (Tanksley et al. 1992). Recently, repeat sequences, which almost exclusively co-localize with centromeres, have been reported. One of these sequences was mapped to a position near TG51 on chromosome 1 (Broun and Tanksley 1996). The map position of *hp-2* and *tri* in a region of low recombination will make map-based cloning difficult.

Mutants for the *PhyB2*, *PhyE* and *PhyF* genes (Hauser et al. 1995; Pratt 1995) have not yet been found. This might be due to the relatively small or specific effects of some members of the phytochrome gene family. Screening of the M_2 population derived from γ radiation of *tri*, *fri* double-mutant seed has produced possible candidates for phytochrome deficiency at these loci (M. Koornneef and R. E. Kendrick personal communication.)

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