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Position of the reduced mycorrhizal colonisation (*Rmc*) locus on the tomato genome map

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Abstract Our research aims to investigate the molecular communication between land plants and arbuscular mycorrhizal (AM) fungi in the establishment of symbiosis. We have identified a mutation in the facultative AM host tomato, which we named *rmc*. Plants that are homozygous for *rmc* no longer host most AM fungi. The mutation also affects the interaction of tomato with root knot nematode and Fusarium wilt. However, the function/s encoded by the intact *Rmc* locus is/are unknown. To clone and sequence the gene or genes that comprise the *Rmc* locus, we have initiated a positional cloning project. In this paper, we report the construction of mapping populations and use of molecular markers from the published genome map to identify the location of *Rmc* on tomato chromosome 8. Nucleotide binding site-leucine rich repeat resistance genes, reported to reside in the same region of that chromosome, provided insufficient differences to develop cleaved amplified polymorphic sequence markers. Therefore, we were unable to map these sequences in relation to *rmc*. Our results potentiate future work to identify the *Rmc* function and to determine the genetic basis for the multiple plant-microbe interaction functions that the *rmc* mutation has defined.

Keywords Arbuscular mycorrhiza · Mutant · Bulked segregant analysis · *Solanum lycopersicum* · Microsatellite · RFLP · NBS-LRR R-genes

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Introduction

Arbuscular mycorrhizal (AM) symbiosis is a fundamental aspect of plant root biology. The evolution of this symbiosis in concert with root evolution means that the genetic pathways governing development of the symbiosis and associated root physiological processes are likely to be core elements of root form and function (Brundrett 2002; Barker et al. 2002). The overall benefit of being an AM symbiont is demonstrated by the extent to which this trait has been maintained throughout the evolution of land plants. Extant non-mycorrhizal plant species are clustered in a few major clades (Tester et al. 1987; Trappe 1987; Fitter and Moyersoen 1996; Wang and Qiu 2006). The evolution of distinct plant clades has probably occurred through major genome reorganisations and increasing ploidy, followed by drift of the duplicated regions to enable the development of novel traits (Blanc and Wolfe 2004). It, therefore, seems likely that extant non-mycorrhizal taxa have survived due to the evolution of distinct and significant alternative traits and may still retain many genetic elements of the pathway/s facilitating AM colonisation.

Although the majority of land plant taxa are able to form AM symbioses, many species, including some important crop plants, are not obligate AM symbionts and can grow well in nutrient-supplemented conditions without an AM fungal partner (Johnson et al. 1997; Smith and Read 1997). Furthermore, plant mutants that are not able to form AM symbioses have been identified, initially amongst nodulation-defective legumes (reviewed by Gianinazzi-Pearson 1996; Peterson and Guinel 2000) and subsequently by direct screening of mutagenised tomato populations (Barker et al. 1998; David-Schwartz et al. 2001, 2003). These mutants are healthy and fecund, indicating that the retention of the AM symbiosis trait through evolution is not a result of genetic overlap with other traits essential for viability. Rather, the trait itself must be of evolutionary benefit even in plants that are not obligate symbionts. Research to identify subtle reproductive advantages of mycorrhizality in these species will be facilitated by the existence of near isogenic (genetically matched) mycorrhizal and non-mycorrhizal lines.

Efforts to identify the function of genes that regulate AM symbiosis began with nodulation mutants. Rapid advances in model legume genomics have provided excellent research tools for positional cloning of genes identified from mutant phenotypes. Stracke et al. (2002) and Endre et al. (2002) each cloned and sequenced an overlapping set of orthologous genes from *Lotus japonicus*, *Pisum sativum*, *Medicago sativa*, *Medicago truncatula* and mutant alleles thereof, providing convincing evidence that the mutated function was a “receptor-like kinase”. A putative Ca^{2+}/calmodulin-dependent protein kinase has also been identified [*DMI3* (*M. truncatula*), orthologous to *Sym9* (*P. sativum*); Lévy et al. 2004; Mitra et al. 2004]. A distinct function is predicted for *DMI1* (*M. truncatula*) and *CASTOR* and *POLLUX* (*L. japonicus*), which encode transmembrane cation channel proteins (Ané et al. 2004; Imaizumi-Anraku et al. 2005). Further investigation using several *L. japonicus* mutants has revealed seven genes required for the establishment of both mycorrhizal and rhizobial symbioses (Kistner et al. 2005). However, the genetic pathways involved in nodulation and mycorrhiza formation do not entirely overlap (Marsh and Schultze 2001; Oldroyd et al. 2005; Zhu et al. 2006), and mutants that are identified without a pre-screen for nodulation phenotype are therefore a valuable asset, enabling more comprehensive understanding of these symbioses.

The first non-legume species in which efforts have been made to identify genes involved in AM colonisation is tomato (Barker et al. 1998; David-Schwartz et al. 2001). Identification of three phenotype classes of mutant in maize has also been reported (Paszkowski et al. 2006).

The reduced mycorrhizal colonisation (*rmc*) mutation in tomato is determined by a single recessive gene (Barker et al. 1998), and the phenotype of mutant plants has been extensively characterised. No innate developmental or reproductive character has been found to differ between *rmc* and its near isogenic parent line, Rio Grande 76R (76R; courtesy of Peto Seed Company, California; Cavagnaro et al. 2004; Bago et al. 2006; Gao 2002). There are three distinct AM root colonisation phenotypes depending upon the AM fungal species involved. These are inability to penetrate the epidermis (Pen^-), inability to colonise the cortex (Coi^-) and slower but successful established symbiosis (Myc^+; Barker et al. 1998; Gao et al. 2001; M Manjarrez and SE Smith, unpublished results). Furthermore, when a successful Myc^+ symbiosis is established, the molecular and physiological aspects of the AM pathway of phosphate uptake is as for non-mutant plants (Poulsen et al. 2005). Interactions of the mutant *rmc* and wild type 76R with some root parasites have also been examined, with a focus on those that in the susceptible interaction penetrate the root cortex but induce minimal defence responses. No differences in susceptibility to *Rhizoctonia* spp. or the bulb and potato aphid *Rhopalosiphoninus latysiphon* (Davidson) were found between *rmc* and 76R (Gao et al. 2006; Barker et al. 2006). However, the *rmc* mutant had an enhanced susceptibility to both races 3 and 4 of Fusarium wilt [*Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) Synd. and Hans] compared with the near isogenic line. Furthermore, a different developmental pattern of the root knot nematode *Meloidogyne javanica* (Treub) Chitwood was observed in *rmc* root organ cultures, compared with 76R (Barker et al. 2006). Positional cloning and characterisation of the *Rmc* locus will enable dissection of its function in AM symbiosis and in pathogen resistance. The work described in this paper led to the new information on the location of *Rmc* on the tomato molecular-genetic map, which will facilitate the overall aim.

Materials and methods

Biological material, growth conditions and mycorrhizal screening

The wild type and mutant tomato lines used in the project were *Solanum lycopersicum* L. cv 76R (previously *Lycopersicon esculentum* Mill. cv 76R) and *S. lycopersicum* L. cv *rmc* (previously *L. esculentum* Mill. cv *rmc*), respectively. Two mapping populations, segregating for AM compatibility, were produced by crossing *rmc* as the female parent with the wild-type *S. pennellii* Corr. cv LA2963 [previously *L. pennellii* (Corr.) D’Arcy cv LA2963]. This accession is a functional AM host that performs well in the conditions used for AM screening (SJ Barker and SE Smith, unpublished results). One F1 individual was back-

crossed as the male to *rmc* to produce BC1 seed (BC mapping population). The same F1 plant was self-pollinated many times to produce a large number of F2 seeds (F2 mapping population).

The AM fungus used for screening the parents and derived mapping populations was *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe WUM23, kindly supplied by L. Abbott, UWA. This species provided a near-unambiguous myc$^-$ phenotype for the mutant. Inoculum of *G. mosseae* was produced in pot cultures of *Trifolium subterraneum* L. grown in University of California Mix Minus Phosphate (Barker et al. 1998). After 12 weeks of growth, the pot cultures were dried and the soil and roots thoroughly mixed. Inoculum therefore contained dried soil, colonised roots, spores and hyphae of *G. mosseae*.

Seedlings were grown in *G. mosseae* inoculum mixed with University of California mix as described previously (Barker et al. 1998), except that pots were contained in Sunbags (Sigma), to reduce water loss and minimise accidental contamination by pests. The potting mix was wet to 15% v/w with deionised water, and pots were watered to weight once per week. Pots were placed in a root-cooling tank at 20°C during December–March (summer), otherwise on a glasshouse bench (cooling commenced at 22°C, with a temperature range of 14–35°C). After 6 weeks growth, plants were carefully removed from the pots and washed to remove the soil mix. Approximately half of each root system was removed from each seedling for the determination of AM colonisation phenotype after trypan blue staining, as described previously (Barker et al. 1998). Seedlings were then transplanted into open pots in a commercial general potting mix and grown on for tissue and seed harvest. The phenotype of myc$^-$ individuals was confirmed by transplanting cuttings, treated with rooting hormone, into fresh pots containing *G. mosseae* inoculum and staining the resultant roots, as above.

DNA extraction

DNA was extracted from 1 g of young leaf tissue that had been snap-frozen in liquid nitrogen, ground into a fine powder and transferred to 10 ml of DNA extraction buffer (1% Na Sarkosyl; 100 mM Trizma base; 100 mM NaCl; 100 mM Na_2SO_3 10 mM EDTA; pH 8.0). Samples were mixed for 5 min before extraction twice with 10 ml Tris-buffered phenol (pH 7.9)/chloroform/iso-amyl alcohol (25:24:1 by volume). Nucleic acids were precipitated by the addition of 1 ml 3 M sodium acetate pH 5.8 and 10 ml isopropanol, pelleted by centrifugation at 4,000 rpm for 5 min, washed with 500 µl 70% ethanol, centrifuged at 13,000 rpm for 3 min, dried in a vacuum centrifuge for 15 min and resuspended overnight in 300 µl Tris-EDTA (TE) buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA). After RNAse A treatment, the DNA was precipitated with ethanol and resuspended in 300 µl TE buffer at 4°C.

Bulked segregant analysis of the BC1 population

A back-cross population was chosen to determine a preliminary map location of the wild type *Rmc* locus because (1) the AM screening process is time- and resource-consuming, (2) *Rmc* is fully dominant so *Rmc*/*Rmc* and *Rmc*/*rmc* individuals cannot be distinguished on the basis of mycorrhizal phenotype; in an F2 population, which is an alternative for mapping work, only 25% of the plants were expected to be the *rmc* phenotype and therefore of unambiguous genotype, (3) 50% of the individuals in a BC population were expected to be *rmc*/*rmc* (myc-), and therefore, more plants from this population could be utilised for preliminary mapping using a bulked segregant analysis approach. This is a method that reduces costs associated with molecular techniques used for genetic mapping (Michelmore et al. 1991).

The concentration of DNA from myc$^-$ BC1 plants was adjusted to 1 mg/ml in TE buffer. The standardised DNAs were then pooled. Initially, bulked samples consisted of DNA from seven individual myc$^-$ BC1 plants; this was later changed to 14 individuals per bulked sample. Pooled DNAs were digested with restriction endonucleases (*Bst*OI, *Dra*I, *Eco*RI or *Eco*RV) and separated on agarose gels, followed by Southern blotting using standard protocols. The same procedure was followed to test putative linked markers, except that BC1 DNAs were not pooled but tested as individuals.

Restriction fragment length polymorphism (RFLP) probes for tomato from the "200 Marker Kit" supplied by SD Tanksley (Cornell University, USA) were chosen so that they spanned the genomic map of tomato (Tanksley et al. 1992) in not more than 30 centiMorgan (cM) intervals and only required one of the four restriction enzymes listed above to detect a polymorphism. With this spacing of markers, there was a high level of probability of detecting the genomic region linked to the trait (Michelmore et al. 1991; Darvasi and Soller 1994). Purified inserts were labelled using α-^{32}P dATP with either a Gigaprime DNA labeling kit (Bresatec) or Prime-A-Gene labeling kit (Promega) according to the manufacturers' protocols. Hybridisation of probes to Southern blots was performed in 10 ml hybridisation solution [0.25 M phosphate buffer, pH 7.2; 1 mM EDTA; 1% bovine serum albumin; 7% sodium dodecyl sulphate (SDS)] overnight at 65°C in a Hybaid mini oven. Membranes were washed twice at room temperature in 2× saline-sodium citrate (SSC), 0.1% SDS, twice in 1× SSC, 0.1% SDS solution and twice in 0.2× SSC, 0.1% SDS. Digital hybridisation images were

obtained by exposure of membranes to a Phosphoimager plate (FujiFilm BAS-IP TR 2040) for 24–36 h that was then read in a Phosphoimager (FujiFilm BAS-2500).

RFLP analysis of the F2 mapping population

Once the general chromosomal location of *Rmc* was identified, finer mapping work was required. For this, the greater resolution capacity of an F2 mapping population (per a given number of plants; Kochert 1994) was desirable. DNA extraction and Southern blotting were performed as described above, except that F2 DNAs were not bulked and all F2s, not just myc- plants, were analysed with some markers.

Microsatellite markers

Primers for two microsatellite markers reported to map in the same region of chromosome 8 as the *rmc* locus (Areshchenkova and Ganal 2002) were obtained. These were: TC948a, 5′TTTTCGCGTTAAGAGATGTT; TC948b, 5′CCGCCATACTGATACGATAG; EST248494a, 5′CTGAAACGAGACAGAGGAAG and EST248494b, 5′AGCTGAGTACGTCTCCCATG. PCR amplification was performed using the Promega PCR core system with an annealing temperature of 48°C and an extension time of 1 min, in a Hybaid PCR express thermal cycler. PCR products were separated on 8% Tris-borate-EDTA polyacrylamide gels, stained with ethidium bromide solution and visualised and recorded under UV light using a Kodak ID digital camera and software.

Development of CAPS markers for NBS-LRR resistance-gene homologues

A published survey of nucleotide binding site-leucine rich repeat resistance gene homologues (NBS-LRR R-genes) in tomato reported a cluster of these sequences in the same region of chromosome 8 as the *Rmc* locus (Pan et al. 2000). Two sequences were chosen for an attempt to refine their map location relative to *Rmc*. Primers were designed from the reported sequences, for an attempt at specific amplification of each gene as follows: Q153F, 5′TGGGAAACGAGACAGAGGAAG; Q153R, 5′ACAGTAGGCAAAACATTGTCTC; Q137F, 5′CTTGCCCCAAAATGGTTC and Q137R, 5′AGGCACAGTACGCAAAAC. Multiple amplified fragments were obtained from both parents of the mapping population from both primer pairs, but fewer fragments were amplified from the Q153 primer pair. These were purified and cloned into pGEM-T plasmid vectors. One colony was selected for each insert size from each tomato line and sequenced (performed by Royal Perth Hospital, Perth, Western Australia) before being identified from the GenBank database using the BlastN function. Cleaved amplified polymorphic sequence (CAPS) markers (Konieczny and Ausubel 1993) were derived from the inspection of the 672-bp sequences amplified by the Q153 primer pair. CAPS primers were CAPS-153f, 5′CGAGGACTTGGCTTCATTTC and CAPS-153r, 5′TCTCTCACATGTTCCCATTCA. PCR conditions were as described for microsatellite markers. Amplification products were cleaved using the enzyme matching the unique enzyme site (*Fok*I; only present in the sequenced *S. pennellii* allele), separated on 1% agarose gels and scored for polymorphism.

Bioinformatics and genetic mapping software

All bioinformatic functions concerned with DNA sequence analysis and primer design were performed using the BioManager suite of software (http://biomanager.angis.org.au). The polymorphism data obtained for all mapping population representatives were compiled with MapManager QTX software (Manly et al. 2001), using the Kosambi mapping function and a search criterion of p=0.05, to derive the most likely genetic map for the *rmc* region.

Results

BC1 population screening for mycorrhizal phenotype

Of the 70 BC1 seeds germinated and subjected to the AM screening process, 31 formed compatible interactions with *G. mosseae* (myc$^+$), 28 showed the AM-deficient phenotype (myc$^-$), 9 did not have sufficient external hyphae in contact with the roots to categorise their mycorrhizal phenotype and 2 died. For the seedlings whose phenotypes were clear and could be scored accurately (59), the segregation of myc$^+$ and myc$^-$ seedlings was the expected 1:1 (p>0.05).

Bulked segregant analysis and preliminary map location of *Rmc*

A total of 28 BC1 plants with the *rmc* phenotype were utilised for bulked segregant analysis. The initial RFLP analysis using four bulks, each of seven myc$^-$ individuals, resulted in identification of a false positive result part way through the screening process. Subsequent examination of individual BC1 DNAs revealed that the apparent co-segregation of the *rmc* allele of the false positive RFLP with the myc$^-$ phenotype was due to uneven grouping of genotypes in the four bulks. To avoid this problem in future hybridisations, the DNAs of the *rmc* phenotype plants were pooled in two larger bulks, each of 14 individual DNAs,

and new membranes were produced. Using the new membranes, the RFLP marker TG41 revealed a skewed polymorphism favouring the *rmc* allele. This marker was tested again using DNA from individual members of the BC1 population. Of the 28 myc$^-$ members screened, only two displayed a heterozygous polymorphism for TG41. The remaining 26 displayed the homozygous *rmc* polymorphism. These data located *Rmc* on chromosome 8. A distance of approximately 7.1 cM between *rmc* and TG41 was calculated from the individual BC1 data.

F2 production and screening of mycorrhizal phenotypes

Of the initial 100 F2 seedlings screened for mycorrhizal compatibility, 16 either did not survive the screening process or did not have enough colonisation pressure from the fungus to display a score-able phenotype. Of the 84 remaining seedlings, 21 displayed a non-mycorrhizal (myc$^-$) phenotype, giving a perfect 3:1 ratio of myc$^+$ to myc$^-$ plants. Of 79 F2 plants in the second batch, only 40 survived the screening process. Of these only two myc$^-$ seedlings were identified; however, the expected ratio of 3:1 for the myc$^+$/myc$^-$ F2 plants used in the study was still accepted (p>0.05). After rescreening the F2 progeny from cuttings, an additional five myc$^-$ progeny were identified from plants with ambiguous phenotypes in the original screening. The final F2 mapping population consisted of 29 myc$^-$ and 52 myc$^+$ individuals.

Fig. 1 Genetic map of the *Rmc* locus. Diagrammatic representation of the mapped positions of seven molecular markers used in this study relative to the *Rmc* locus on chromosome 8 of tomato. All distances in cM were determined in this study. Relative order and spacing of markers are consistent with the published map (Tanksley et al. 1992)

RFLP mapping of the *Rmc* region

To discover in which direction from TG41 the *rmc* locus lay, the RFLP markers TG41, CT92, TG349 and TG302 were used to screen the 29 myc$^-$ F2 plants, and these revealed a variable number of recombination events with *rmc*. These results indicated that the *rmc* locus was positioned below TG41 (oriented according to the Tanksley map, Fig. 1) and also below TG302, the most distant marker from TG41 tested to this point. When two additional markers positioned below TG302 (CT88 and TG330) were tested, the results indicated that markers had now been placed both above and below the *Rmc* locus. The remaining F2 plants (myc$^+$ members of the population) were scored with all markers to determine the position of the *rmc* locus relative to TG302 and CT88, as there was insufficient information in the myc$^-$ members alone to determine if CT88 was positioned above or below the locus. One additional RFLP marker, TG69, was positioned between the two closest flanking markers (TG302 and CT88; Fig. 1) on the Tanksley map. The end sequences of the CT69 clone were obtained from the SolGenes database (http://grain.jouy.inra.fr/cgibin/webace/webace?db=solgenes). Primers were designed for these sequences, and the marker fragment was amplified from tomato genomic DNA. However, the resulting fragment did not provide a polymorphism that could be scored (data not shown), and therefore, this marker could not be included in the study.

Markers both linked to and flanking the *Rmc* locus had been identified at this point in the investigation. The markers retained the same order and relative spacing as presented on the published Tanksley map and gave distances of 10.0 cM for the TG302-*Rmc* interval and 1.4 cM for the *Rmc*-CT88 interval. To refine the genetic map for the *Rmc* region further, alternative markers were sought.

Microsatellite markers and closest flanking markers for *Rmc*

From the available literature, two microsatellite markers were identified that were potentially linked to *Rmc*. Amplification of the microsatellite TC948 failed to reveal a polymorphism between the parental genotypes of the population. However, PCR using primers for microsatellite EST248494 identified a co-dominant polymorphism be-

tween the parental genotypes that could be easily scored. When this marker was used to screen the myc^{-} members of the F2 population, no recombination events between *rmc* and marker were detected. Only homozygous *rmc*-type alleles were found, indicating very tight linkage of this microsatellite marker to the *Rmc* locus. When the marker was used to screen the myc^{+} members of the F2 population, the microsatellite was found to lie at a distance of only 0.7 cM from *Rmc*, in the direction of the RFLP marker TG302. Only one of the F2 individuals displayed a recombination event between the marker and gene. The completed molecular genetic map of the *Rmc* locus was formed by compiling all polymorphism information on both mapping populations, and is shown in Fig. 1.

Development of CAPS markers for R gene homologues in the *Rmc* region

During the development of the molecular genetic map of the *Rmc* region, we were mindful of the possibility that *Rmc* might encode a gene of known function in plant–microbe interaction. Therefore, we investigated the possibility that the locus might include R-gene homologues that had been reported to be positioned in the same region of tomato chromosome 8. Primers to amplify two R gene homologues (called Q153 and Q137) were designed as described in "Materials and methods". PCR on both parents of the F2 population (*rmc* and LA2963) as well as the non-mutant 76R (the parent line of *rmc*) gave multiple fragments, as might be expected for PCR analysis of a large multi-gene family. However, as a map location had been reported for these members of the family, we decided to persist with the analysis and see if we could refine our primers for more specific amplification. Unfortunately, there was insufficient variation in the sequences obtained to design a pair of primers that would amplify only one unique *S. pennellii* and *rmc* fragment, which would be necessary to convert these sequences into functional CAPS markers (data not shown). Therefore, this attempt to map the R gene homologues around the *Rmc* locus was discontinued.

Discussion

The bulked segregant analysis technique (Michelmore et al. 1991), in combination with a BC1 population prepared from the *rmc* mutant and a wild-type *S. pennellii* LA2963, was successfully used to identify an RFLP marker linked to the *rmc* mutation in tomato. This provided a general location for *Rmc* on chromosome 8 and allowed a more detailed mapping strategy for the *Rmc* locus to be developed, which was economical of time and resources. To progress the project towards the ultimate goal of positional cloning of the gene, it was necessary to further define the *Rmc* region of chromosome 8. A larger segregating population was required for the study of the chromosomal region surrounding the *Rmc* locus because including more individuals in a segregation test increases the accuracy of the mapping data and the resolution of the genetic map (Leyser and Chang 1996). The F2 population had one important advantage over the BC1 population; it potentially had a much higher recombination frequency, providing more mapping information than a BC1 population, for a given number of plants (Kochert 1994).

Analysis of the F2 population derived from a cross between *rmc* and *S. pennellii* LA2963 gave a molecular map of the *Rmc* locus (Fig. 1). On one side of the *Rmc* locus, the RFLP marker CT88 (Tanksley et al. 1992) was linked to *Rmc* at a distance of 1.4 cM; however, the distance between the gene and the other flanking RFLP marker, TG302, was 10 cM. The position of the microsatellite EST248494, linked to *Rmc* on the opposite side to CT88, was extremely fortuitous. The paper in which this marker was identified only described 20 microsatellite markers (Areshchenkova and Ganal 2002). At the time this work was performed, there were no published, publicly available microsatellite maps for the tomato genome from which to obtain additional markers for this project, although future editions of the Solanaceae Genomics Network map (http://www.sgn.cornell.edu/) promise to incorporate microsatellite information. It should be noted that, in both mapping populations, *rmc* segregated according to expectations as a single locus mutation (Barker et al. 1998).

An attempt was made to create a CAPS marker from previously published R-gene sequences (Pan et al. 2000) although not successful (data not shown). The R-gene sequences were incorporated into the study primarily because of their reported position on chromosome 8 near the *Rmc* locus. It was also potentially interesting to include the R-gene homologues because these genes code for proteins, which are suggested to act as receptors for specific microbial ligands (Staskawicz et al. 1995), and it has been suggested that there are commonalities between the gene pathways for microbial defence and symbiosis (Parniske 2000). If this is the case, then it is possible that *Rmc* could code for a similar sequence. Furthermore, a receptor-like kinase gene, *SYMRK* from *L. japonicus*, is reported to play a role in both arbuscular mycorrhizal and rhizobial symbioses (Stracke et al. 2002) and has a basic structure similar to that of the rice *Xa21* resistance gene (Song et al. 1995) suggesting a role in both symbiotic and pathogenic interactions for these receptors.

The R-gene homologue sequences cloned from the parent tomato lines used in this study displayed an extremely high sequence similarity, so much so that it was not possible to reliably design a primer pair that would only

amplify one of the sequences rather than a group of very similar sequences. This finding was not unexpected because extensive gene clusters of R-gene sequences are common in plants and reflect their role in creating new specificity to plant pathogens, through gene duplication, recombination and chromosomal duplication (Lehmann 2002).

The *rmc* mutation has also been shown to result in increased susceptibility to races 3 and 4 of Fusarium wilt and increased reproduction of root knot nematode in root organ culture (Barker et al. 2006). A locus conferring tolerance to race 3 of Fusarium wilt was separately reported to reside in the region of *Aps-2* (Bournival et al. 1989), which is adjacent to TG-41 on the tomato genome map of chromosome 8 (Tanksley et al. 1992). From our results, the two loci are either closely linked or are the same gene. Positional cloning of the *Rmc* locus will enable us to address this question and will provide important functional information on how *Rmc* functions in facilitating AM symbioses.

Although the size of the population used for this experiment was relatively small, limiting the power of the analysis, we have now established a map position for *rmc*, flanked by the markers CT88 and EST248494 on chromosome 8 of the tomato map. Our results allow for future fine mapping of the interval using a much larger population and facilitation of the ultimate goal of cloning *rmc*.

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