Theor Appl Genet (2002) 105:160–166
DOI 10.1007/s00122-002-0909-1

M.E. Humphry · V. Konduri · C.J. Lambrides
T. Magner · C.L. McIntyre · E.A.B. Aitken · C.J. Liu

Development of a mungbean (*Vigna radiata*) RFLP linkage map and its comparison with lablab (*Lablab purpureus*) reveals a high level of colinearity between the two genomes

Received: 17 May 2001 / Accepted: 8 October 2001 / Published online: 23 May 2002

Abstract A genetic linkage map of mungbean (*Vigna radiata*, 2n = 2*x* = 22) consisting of 255 RFLP loci was developed using a recombinant inbred population of 80 individuals. The population was derived from an inter-subspecific cross between the cultivated mungbean variety 'Berken' and a wild mungbean genotype 'ACC 41' (*V. radiata* subsp. *sublobata*). The total length of the map, which comprised 13 linkage groups, spanned 737.9 cM with an average distance between markers of 3.0 cM and a maximum distance between linked markers of 15.4 cM. The mungbean map was compared to a previously published map of lablab (*Lablab purpureus*, 2n = 2*x* = 24) using a common set of 65 RFLP probes. In contrast to some other comparative mapping studies among members of the Fabaceae, where a high level of chromosomal rearrangement has been observed, marker order between mungbean and lablab was found to be highly conserved. However, the two genomes have apparently accumulated a large number of duplications/deletions after they diverged.

Keywords Mungbean · Lablab · Comparative mapping · Synteny · Colinearity

Introduction

Comparative mapping of plant genomes has become an important branch of modern genomics and has facilitated further understanding of the mechanisms involved in the evolution of most plant species. In addition, comparative mapping experiments have been used to better characterise important genes in those crops that are difficult to work with at the molecular level or that have incomplete maps. This has been achieved by saturating the genomes of these species in regions of interest with markers from homoeologous chromosomal sections of other genetically similar species to increase the efficacy of existing markers. Using this strategy, resistance genes have recently been mapped in soybean (*Glycine max*) using clones from other members of the Fabaceae (Concibido et al. 1996), as well as in sugarcane (*Saccharum* spp.) using clones from the Poaceae family (Asnaghi et al. 2000). Orthologous seed-weight genes have also been located in mungbean (*Vigna radiata*), cowpea (*Vigna unguiculata*) and soybean (Fatokun et al. 1992; Maughan et al. 1996) using a similar approach.

Understandably, a large proportion of comparative mapping research has centred on members of the Gramineae due to their economic importance. Interestingly, this research has revealed that many species within this family have retained highly conserved genome structures and colinearity across both genus and tribe boundaries, despite vast differences in genome size and ploidy level (Helentjaris 1993; Gale and Devos 1998; Devos and Gale 2000). However, in the Solanaceae it appears that while some reproductively isolated members of this family share a substantial gene order and repertoire (Bonierbale et al. 1988; Tanksley et al. 1992), others have undergone significant chromosomal rearrangement (Tanksley et al. 1988; Helentjaris 1993). Similar results have been obtained in the Crucifereae from the comparative mapping of *Brassica* species (Lagercrantz and Lydiate 1996; Gale and Devos 1998).

Within the Fabaceae, there has been comparatively little research into genome evolution, but the emerging picture is that the level of genome conservation in this family may not be as universal as in the Gramineae. Rather, there appear to be several groups of species with conserved genomes within the family. In early reports on comparative mapping in legumes, a high level of conser-

Communicated by P. Langridge

M.E. Humphry · V. Konduri · C.J. Lambrides · T. Magner
C.L. McIntyre · C.J. Liu (✉)
CSIRO Division of Plant Industry, Indooroopilly,
Queensland 4068, Australia
e-mail: Chunji.liu@pi.csiro.au
Tel.: +61-7-3214-2200, Fax: +61-7-3214-2288

M.E. Humphry · E.A.B. Aitken
Department of Botany, University of Queensland,
St. Lucia, Queensland 4072, Australia

vation between the cowpea and mungbean (both members of the Phaseoleae tribe), and mungbean and common bean (*Phaseolus vulgaris*) genomes was reported (Menancio-Hautea et al. 1993a; Boutin et al. 1995). However, investigations into synteny between both common bean and soybean, as well as Viceae tribe members pea (*Pisum sativum*) and lentil (*Lens* spp.), showed that homology was retained only in dispersed blocks throughout their genomes (Weeden et al. 1992; Boutin et al. 1995). Similarly, Kaga et al. (2000) recently reported that, within the genus *Vigna*, small insertions, deletions and rearrangements have resulted in the divergence of several species that have become reproductively incompatible.

Despite the work carried out on the Fabaceae, research into mungbean genetics and evolution is not as advanced as in many other species. Several linkage maps of mungbean have been constructed (Menancio-Hautea et al. 1993b; Lambrides et al. 2000) upon which most marker research into this crop has been based, but they do not provide the same level of genome saturation seen in many other species. In this study, we attempted to produce a mungbean linkage map with a more extensive genome coverage by utilising clones from three separate sources: (1) a set of probes covering an existing mungbean map (Menancio-Hautea et al. 1993b), (2) a set of probes covering a linkage map of lablab (*Lablab purpureus*; Konduri et al. 2000), and (3) new mungbean clones constructed in this study. In addition to providing greater genome coverage, the use of the lablab probes would allow the investigation of genetic relationships between these two members of the Phaseoleae tribe.

Materials and methods

Mungbean plant material

A subset of 80 individuals from a recombinant inbred (F_8) population (Lambrides et al. 2000) of mungbean was used in this study. The population was derived from an inter-subspecific cross between a commercial cultivar, 'Berken' (*V. radiata* ssp. *radiata*), and a wild mungbean genotype, 'ACC 41' (*V. radiata* ssp. *sublobata*). For the collection of leaf material, the parents were grown in glasshouses in 1-litre pots at St. Lucia, Queensland, and the RI population was grown in the field at Samford, Queensland. Ten individuals from each line in the population were grown to ensure that adequate leaf material was available for DNA analysis. Leaf material was periodically harvested from both the parents and progeny, and frozen in liquid nitrogen prior to freeze-drying in a Dynavac FD-2 freeze drying unit, ready for DNA extraction.

RFLP clones

Clones from three sources were used in the study. (1) Eighty three clones from an existing mungbean map (Menancio-Hautea et al. 1993b). Sixty five of these were mungbean clones and were named with the prefix mc, mgM, mgQ or mgR; the remaining 18 were from cowpea [*V. unguiculata* (L.) Walpers] and were prefixed by either cgO or cgP. These mungbean and cowpea clones were a kind donation from Dr. N. D. Young, University of Minnesota, St. Paul, Minnesota, USA. (2) Seventy six clones which form a skeleton map of lablab (Konduri et al. 2000). These clones were prefixed by LpCS. (3) New mungbean *Pst*I clones constructed using total genomic DNA from leaves of mungbean cultivars 'Berken' or the introduction line 'CPI 109897'. The protocol employed to create these clones was as described in Liu et al. (1994) with the exceptions that the genomic DNA was purified with a QIAEX II Agarose-Gel Extraction Kit (QIAGEN) and pBluescript II KS^+ was used as the cloning vector. Amplification and analysis of probes to detect for the presence of highly repetitive sequences has been previously described (Konduri et al. 2000). These clones were arbitrarily designated VrCS1–VrCS389 to indicate the source of the clone/probe (Vr for *V. radiata*), the institution where the research was carried out (CS for CSIRO) and the clone number.

Mungbean DNA extraction and RFLP analysis

Freeze-dried leaf material was crushed using ball bearings in a Shatterbox (Spex Industries) and DNA was isolated using a protocol modified from Dellaporta et al. (1983) as described in Lambrides et al. (2000), with the exception that precipitated DNA was looped and not pelleted. Protocols for restriction enzyme digestion, electrophoresis, Southern transfer, probe labelling and filter hybridisations were carried out as described in Sharp et al. (1988), with the exceptions that Hybond N^+ nylon membrane (Amersham) was used and 4 mM spermidine was employed in restriction digests when required. Membranes were washed twice in 2 × SSC and 0.1% SDS, and then once in 1 × SSC and 0.1% SDS for 15 mins each at 60 °C prior to exposure on Kodak X-Omat film (Kodak Eastman). Membranes were then stripped for re-use in a boiling solution of 0.1 × SSC and 0.5% SDS for 30 mins.

Ten restriction enzymes, *Bam*HI, *Bst*OI, *Dra*I, *Eco*RI, *Eco*RV, *Hae*III, *Hin*dIII, *Kpn*I, *Xba*I and *Xho*I (Gibco BRL), were used to assay the parental genotypes for polymorphic probe-enzyme combinations. Those probe-enzyme combinations that showed a clear polymorphism between the two parental genotypes were used in the subsequent mapping experiments.

RFLP data analysis

Each individual was scored according to whether they inherited a maternal or paternal allele for all polymorphic RFLP probes except those that were either heterozygous or ambiguous, which were scored as missing data. Probes that gave multiple bands where allelism could not be determined were scored by the presence or absence of bands. The data was then error checked manually to ensure fidelity of the results. Linkage analysis was carried out using the Map Manager QTX package Version 0.18 (Manly and Olson 1999) with $\alpha = 1 \times 10^{-6}$ (probability of a Type I error) using the Kosambi map function and allowing for segregation distortion. Rare-double crossover events were identified using the Map Manager program and potentially questionable data were manually re-checked for errors. Tentative linkage between groups was analysed using the Find Links command, and those with a LOD ≥ 4.0 were considered linked. Finally, loci deviating from the expected 1:1 ratio were identified using the chi-square test.

Analysis of lablab data and comparative mapping

All collection of plant material, DNA extraction and Southern hybridisations used in constructing the lablab linkage map was previously performed by Konduri et al. (2000). All additional analysis of linkage order was carried out using Map Manager QTX and the comparison between the mungbean and lablab linkage maps was undertaken manually, based on the location of common markers.

Results

New mungbean *Pst*I clones

Inserts from 375 putative recombinant clones were amplified by PCR using M13 forward and reverse primers and screened with *Hae*III-digested total genomic DNA. Ninety one (24.3%) of these gave strong signals, indicating they would detect high-copy sequences and were thus not used for further analysis. The remainder were then screened against the two parental genotypes. These probes detected either single fragments or up to five separate fragments. Segregation of these fragments was then determined in the progeny filters.

Polymorphism between the parents of the mapping population

Of the 284 remaining low- and single- copy mungbean probes, 246 were randomly selected and analysed for polymorphic probe-enzyme combinations. Initial screening of the two parents indicated that all but two polymorphisms were able to be detected by using six of the ten enzymes (*Bst*0I, *Dra*I, *Eco*RI, *Eco*RV, *Hae*III and *Hin*dIII). One hundred and twenty eight (52.0%) of the 246 probes screened detected polymorphisms between the two parents. Banding patterns from the remainder were either monomorphic or could not be resolved easily. Two additional probes detected polymorphisms between the parents but all of the progeny showed the maternal banding patterns, suggesting that either there was a degree of heterozygosity in the parents or that the clones may have been derived from cytoplasmic DNA.

Of the 83 probes from the previous mungbean map, 38 (45.8%) detected polymorphisms between the two parents. Of the 76 lablab probes, 48 (63.2%) detected polymorphisms with at least one of the above six enzymes.

The mungbean linkage map

The 214 probes identified as being polymorphic detected 260 loci. One hundred and eighteen of these loci (45.4%) were derived from probes that detected single-copy DNA sequences. The remaining 96 probes detected either two or more non-allelic fragments, or monomorphic fragments with one or more allelic fragments. Two hundred and fifty five of these loci were assigned to 13 linkage groups varying in length from 8.7 cM to 100.7 cM. This spanned a total genetic distance of 737.9 cM with an average distance between markers of 3.0 cM and a maximum distance of 15.4 cM. Five markers (LpCS51, VrCS200, VrCS361, VrCS362 and VrCS215-3) remained unlinked. Ten of the linkage groups were assigned letters based on their order on a previously published mungbean map (Menancio-Hautea et al. 1993b). The remaining three linkage groups (E, L and M) could not be unambiguously assigned to corresponding groups based on the co-location of common markers. They were assigned an order from the largest to the smallest map distance (see Fig. 1).

Segregation ratios at 80 (30.8%) of the 260 loci showed a deviation from the expected 1:1 ratio, 27 (10.4%) at a significance level of 0.05 and 53 (20.4%) at 0.01. Distortion was observed for all of the five unlinked markers and distorted markers were found on ten of the thirteen linkage groups (Fig. 1). Only three of these loci were skewed in the direction of the wild male parent ‘ACC 41’ (two at a significance level of 0.01) and all of these were localised to linkage group B.

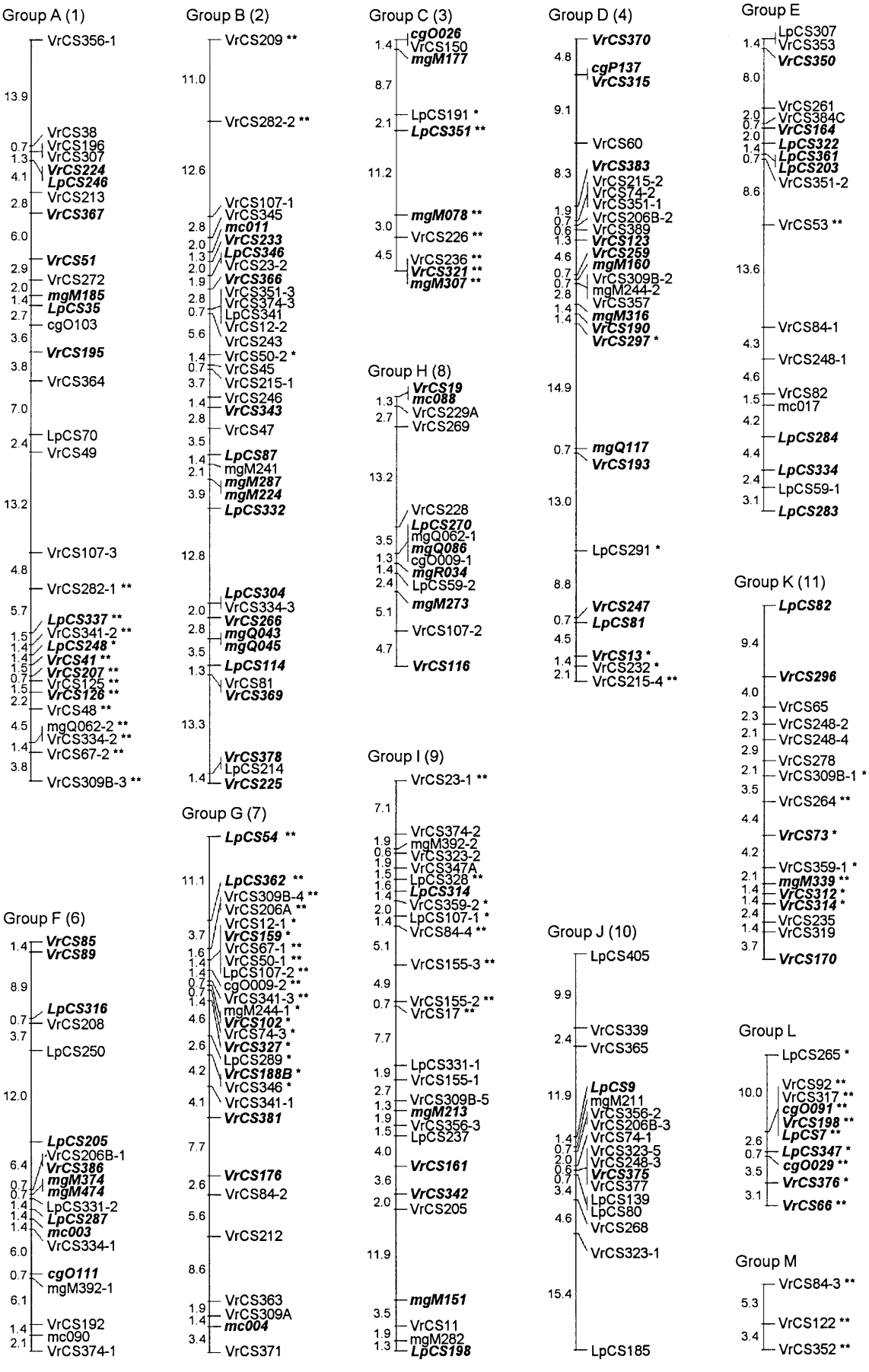
Comparison of mungbean and lablab linkage maps

Sixty five common probes from the lablab, mungbean and cowpea libraries were used to compare the mungbean and lablab linkage maps. These mapped to 68 and 72 loci on the lablab and mungbean maps, respectively. Probes mgM185 and mgM213 detected a single locus each in mungbean but two loci each in lablab. Conversely, probes mgM244, LpCS107, LpCS331, mgM392, mgQ062, cgO009 and LpCS59 detected a single locus each in lablab but at least two loci each in mungbean. Another probe, mc090, detected a single locus in mungbean and two in lablab. However, in addition to the single locus mapped, it also produced a monomorphic fragment in mungbean. This monomorphic fragment may or may not represent the existence of a second locus in mungbean.

Several other probes detected one or more monomorphic fragment(s) in addition to one or more mapped loci in either or both genomes. Most of these loci mapped to their expected positions and were confirmed by their linkage to surrounding markers common to both genomes. The only exceptions were LpCS237, LpCS198 and mgM211, which were mapped to comparatively different chromosomal regions in the two genomes.

Two other probes, LpCS289 and LpCS307, each detected a single locus and a monomorphic fragment in both genomes. The locations of these loci were not able to be confirmed by linkage to any surrounding markers. Thus, the relationship between the two pairs of linkage groups (E and 17, G and 4) based on these markers is only tentative (Fig. 2).

Fig. 1 A mungbean (*V. radiata*) linkage map constructed using RFLP markers. Linkage groups are ordered primarily based on their similarity to a previously published mungbean map (*numbers in brackets* indicate previous ordering; see text). The *long vertical bars* indicate linkage groups and *small horizontal bars* indicate locus positions. Genetic distances (cM) are located to the left of the linkage groups and *locus names* are listed to the right. Marker names containing *hyphens followed by a number* indicate different non-allelic loci detected by single probes and those followed by the *letters A or B* indicate different amplification products from the same clone. Markers showing skewed segregation away from the predicted 1:1 ratio are indicated by an *asterisk* (*) if deviating at a significance level of 0.05, and a *double asterisk* (**) if deviating at a 0.01 significance level. Markers originating from clones that detected single loci are shown in *bold italics* ▶


Group A (1)
VrCS356-1
13.9
VrCS38
0.7 VrCS196
1.3 VrCS307
4.1 VrCS224
LpCS246
2.8 VrCS213
VrCS367
6.0
VrCS51
2.9 VrCS272
2.0 mgM185
1.4 LpCS35
2.7 cgO103
3.6
VrCS195
3.8 VrCS364
7.0
LpCS70
2.4 VrCS49
13.2
VrCS107-3
4.8 VrCS282-1 **
5.7
LpCS337 **
1.5 VrCS341-2 **
1.4 LpCS248 *
1.4 VrCS41 **
1.5 VrCS207 **
0.7 VrCS125 **
1.5 VrCS126 **
2.2 VrCS48 **
4.5 mgQ062-2 **
VrCS334-2 **
1.4 VrCS67-2 **
3.8
VrCS309B-3 **
Group B (2)
VrCS209 **
11.0
VrCS282-2 **
12.6
VrCS107-1
VrCS345
2.8 mc011
2.0 VrCS233
1.3 LpCS346
2.0 VrCS23-2
1.9 VrCS366
2.8 VrCS351-3
VrCS374-3
0.7 LpCS341
VrCS12-2
5.6
VrCS243
1.4 VrCS50-2 *
0.7 VrCS45
3.7 VrCS215-1
1.4 VrCS246
VrCS343
2.8
VrCS47
3.5
1.4 LpCS87
2.1 mgM241
mgM287
3.9 mgM224
LpCS332
12.8
LpCS304
2.0 VrCS334-3
VrCS266
2.8 mgQ043
mgQ045
3.5
1.3 LpCS114
VrCS81
VrCS369
13.3
VrCS378
1.4 LpCS214
VrCS225
Group C (3)
cgO026
1.4 VrCS150
mgM177
8.7
2.1 LpCS191 *
LpCS351 **
11.2
mgM078 **
3.0 VrCS226 **
4.5
VrCS236 **
VrCS321 **
mgM307 **
Group D (4)
VrCS370
4.8
cgP137
VrCS315
9.1
VrCS60
8.3 VrCS383
VrCS215-2
VrCS74-2
1.9 VrCS351-1
0.7 VrCS206B-2
0.6 VrCS389
1.3 VrCS123
4.6 VrCS259
mgM160
0.7
VrCS309B-2
0.7 mgM244-2
2.8 VrCS357
1.4 mgM316
1.4 VrCS190
VrCS297 *
14.9
0.7 mgQ117
VrCS193
13.0
LpCS291 *
8.8
VrCS247
0.7 LpCS81
4.5
1.4 VrCS13 *
VrCS232 *
2.1 VrCS215-4 **
Group E
LpCS307
1.4 VrCS353
VrCS350
8.0
VrCS261
2.0 VrCS384C
0.7 VrCS164
2.0 LpCS322
1.4 LpCS361
0.7 LpCS203
VrCS351-2
8.6
VrCS53 **
13.6
VrCS84-1
4.3
VrCS248-1
4.6
1.5 VrCS82
mc017
4.2
LpCS284
4.4
LpCS334
2.4 LpCS59-1
3.1 LpCS283
Group F (6)
1.4 VrCS85
VrCS89
8.9
LpCS316
0.7 VrCS208
3.7
LpCS250
12.0
LpCS205
6.4 VrCS206B-1
VrCS386
0.7 mgM374
0.7 mgM474
1.4 LpCS331-2
1.4 LpCS287
1.4 mc003
6.0 VrCS334-1
0.7 cgO111
mgM392-1
6.1
1.4 VrCS192
mc090
2.1 VrCS374-1
Group G (7)
LpCS54 **
11.1
LpCS362 **
VrCS309B-4 **
VrCS206A **
VrCS12-1 *
3.7 VrCS159 *
1.6 VrCS67-1 **
1.4 VrCS50-1 **
1.4 LpCS107-2 **
0.7 cgO009-2 **
0.7 VrCS341-3 **
1.4 mgM244-1 *
4.6 VrCS102 *
VrCS74-3 *
2.6 VrCS327 *
LpCS289 *
4.2 VrCS188B *
VrCS346 *
4.1 VrCS341-1
VrCS381
7.7
VrCS176
2.6 VrCS84-2
5.6
VrCS212
8.6
1.9 VrCS363
1.4 VrCS309A
mc004
3.4
VrCS371
Group H (8)
VrCS19
1.3 mc088
2.7 VrCS229A
VrCS269
13.2
VrCS228
LpCS270
3.5 mgQ062-1
mgQ086
1.3 cgO009-1
1.4 mgR034
2.4 LpCS59-2
mgM273
5.1
VrCS107-2
4.7
VrCS116
Group I (9)
VrCS23-1 **
7.1
VrCS374-2
1.9 mgM392-2
0.6 VrCS323-2
1.9 VrCS347A
1.5 LpCS328 **
1.6 LpCS314
1.4 VrCS359-2 *
2.0 LpCS107-1 *
1.4 VrCS84-4 **
5.1
VrCS155-3 **
4.9
0.7 VrCS155-2 **
VrCS17 **
7.7
1.9 LpCS331-1
VrCS155-1
2.7
1.3 VrCS309B-5
mgM213
1.9 VrCS356-3
1.5 LpCS237
4.0
VrCS161
3.6
VrCS342
2.0 VrCS205
11.9
mgM151
3.5
VrCS11
1.9 mgM282
1.3 LpCS198
Group J (10)
LpCS405
9.9
2.4 VrCS339
VrCS365
11.9
LpCS9
mgM211
VrCS356-2
VrCS206B-3
1.4 VrCS74-1
0.7 VrCS323-5
2.0 VrCS248-3
0.6 VrCS375
0.7 VrCS377
3.4
LpCS139
LpCS80
4.6 VrCS268
VrCS323-1
15.4
LpCS185
Group K (11)
LpCS82
9.4
VrCS296
4.0
VrCS65
2.3 VrCS248-2
2.1 VrCS248-4
2.9
VrCS278
2.1 VrCS309B-1 *
3.5
VrCS264 **
4.4
VrCS73 *
4.2
VrCS359-1 *
2.1 mgM339 **
1.4 VrCS312 *
1.4 VrCS314 *
2.4
VrCS235
1.4 VrCS319
3.7
VrCS170
Group L
LpCS265 *
10.0
VrCS92 **
VrCS317 **
cgO091 **
VrCS198 **
2.6 LpCS7 **
0.7 LpCS347 *
3.5 cgO029 **
VrCS376 *
3.1
VrCS66 **
Group M
VrCS84-3 **
5.3
VrCS122 **
3.4
VrCS352 **

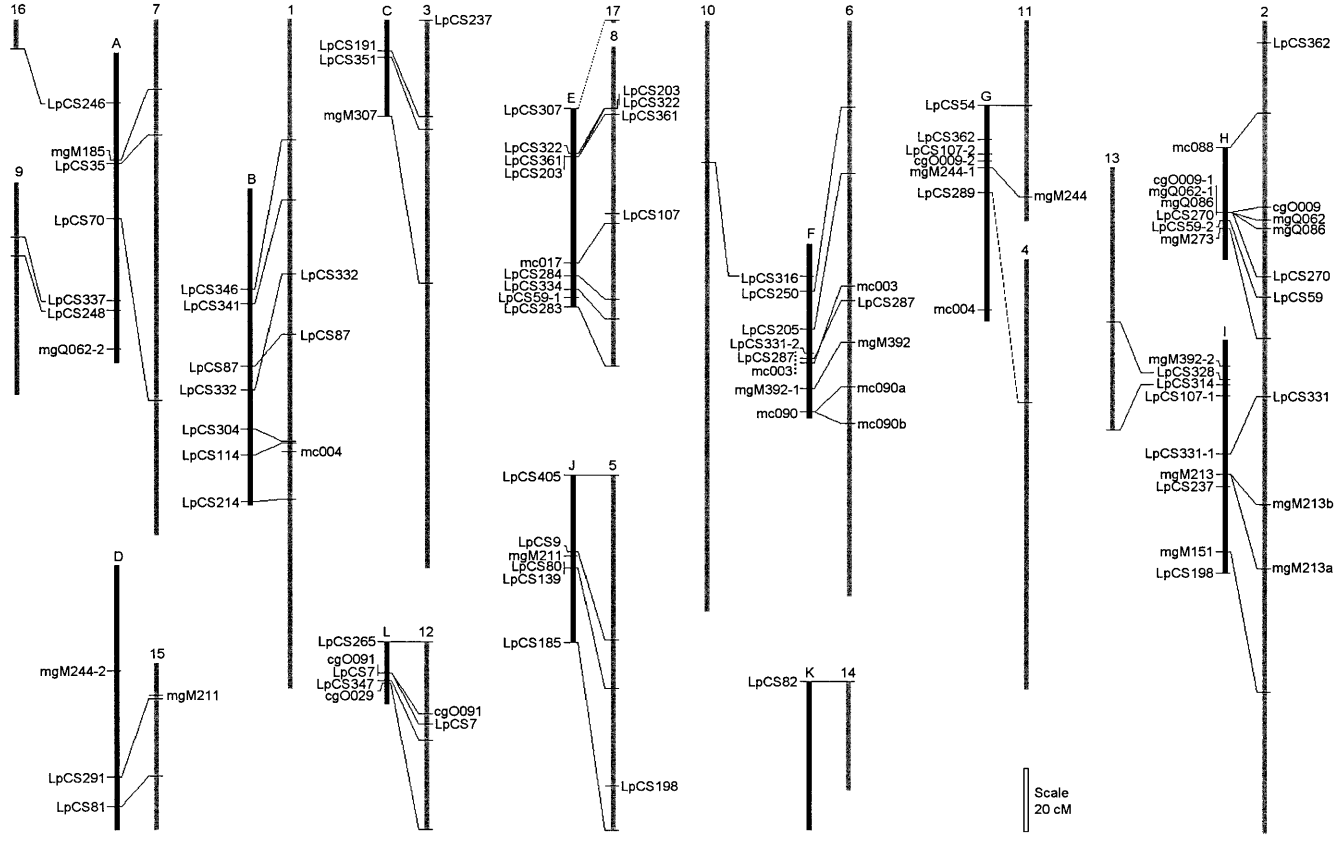


Fig. 2 Comparative linkage maps of the mungbean (*V. radiata*) and lablab (*L. purpureus*) genomes. Mungbean linkage groups are shown as *black vertical lines* capped by letters and lablab linkage groups as *grey vertical lines* capped by numbers. The *dotted vertical line* to the right of markers mc003 and LpCS287 in linkage group F of mungbean indicates the ambiguous marker order of these two loci positions. The *dotted line* connecting marker LpCS289 between group G of mungbean and 4 of lablab, and LpCS307 between E and 17, indicates that non-single-copy loci were used and the results were not confirmed by surrounding loci (see text). Both linkage groups are drawn to scale

Of all the common markers mapped, map order for two pairs appeared reversed between the two species (Fig. 2). However, marker order for one pair (mc003 and LpCS287 in linkage group F) was able to be reversed in mungbean without significantly affecting the LOD scores. Similarly, two probes (LpCS362 and mc004) that detected single loci in both mungbean and lablab mapped to non-corresponding genomic regions. These discrepancies in map locations indicate possible chromosomal rearrangements between the two species.

Twelve mungbean and 17 lablab linkage groups were compared using the 65 common markers. Only linkage group M from mungbean was not represented due to a lack of common markers. The linkage order of markers was clearly highly conserved between the two species allowing a detailed examination of corresponding linkage groups. In addition to the presence of large conserved linkage blocks between mungbean and lablab, the results indicated that lablab groups 7, 9 and 16 could be consolidated into one linkage group, and 6 and 10, 8 and 17 and 4 and 11 into three others (Fig. 2). Similarly, groups H and I of mungbean may be from a single chromosome based on their relationship to lablab group 2. Considering the high conservation of marker/gene order, we expected that all of the markers on groups H and I of mungbean would map to group 2 of lablab. However, instead of mapping to the middle of group 2, markers LpCS328 and LpCS314 mapped to group 13 of lablab (Fig. 2).

Discussion

*Pst*I small insert clones

Burr et al. (1988) demonstrated that cleaving DNA with a methylation-sensitive enzyme such as *Pst*I would result in a genomic library enriched with probes that would detect low-copy sequences. A similar result was obtained in this study on mungbean. However, the percentage of clones detecting the high-copy sequences observed here was considerably higher than that observed in other members of the Fabaceae (Vallejos et al. 1992; Menancio-Hautea et al. 1993b; Konduri et al. 2000). The cause of this increase in high-copy probes obtained in the mungbean library described here is unknown. It would be expected that mungbean should have a relatively low level of highly repetitive sequences as it has a relatively small genome (approximately 580 million base pairs; Arumuganathan and Earle 1991).

The mungbean linkage map

In addition to the new mungbean probes used in this study, framework RFLP probes covering mungbean (Menancio-Hautea et al. 1993b) and lablab (Konduri et al. 2000) maps were also used in constructing this new mungbean map. Thus, we expected a map with more extended genome coverage than the previously published mungbean RFLP maps. Despite the possible increase in genome coverage, the number of linkage groups (13) obtained in this study is still larger than the mungbean basic chromosome number ($x = 11$) and there are still five unlinked markers.

The mungbean linkage map presented here, with a distance of 737.9 cM between linked markers, is significantly smaller than the map published by Menancio-Hautea et al. (1993b) of 1,570 cM. The most likely reason for the differences in size between the two maps is that, although both were inter-subspecific crosses between ssp. *radiata* and ssp. *sublobata*, the population used here was derived from more divergent parents than that of Menancio-Hautea et al. (1993b). Previous studies have indicated that maps generated from wider crosses, although more likely to detect polymorphism, result in lower recombination rates than those between adapted varieties and therefore smaller map sizes (Paterson et al. 1988; Menancio-Hautea et al. 1993a; Liu et al. 1996). However, factors other than the distance between the parents of the cross may also be involved. The choice of parents as male versus female (Gadish and Zamir 1987; de Vicente and Tanksley 1991) as well as environmental effects (Robertson 1984) have also been shown to be involved in influencing rates of crossovers within crosses, and thus map sizes.

A comparison of the mungbean and lablab genomes

We found in this study that lablab and mungbean probes cross-hybridised easily. As a consequence, the mungbean linkage map was able to be compared to the previously published map of lablab. The results showed that mungbean shares a high level of homology with lablab. The only alteration to marker order was observed between LpCS332 and LpCS87 on groups B of mungbean and 1 of lablab. This observation suggests that the two species may differ by an inversion at this genomic region. However, this possibility was not confirmed by any surrounding markers, such as observed in the comparison of the mungbean map to the interspecific cross of rice bean (*Vigna umbellata*) and adzuki bean (*Vigna angularis*; Kaga et al. 2000) where several regions of chromosomal rearrangements were evident.

It is surprising that mungbean appears to share a higher level of homology with lablab than with some other members of the genus *Vigna* (Kaga et al. 2000). Mungbean and lablab are thought to be more phylogenetically distant and have different basic chromosome numbers (11 and 12 respectively). However, despite their very similar marker orders, the two genomes have apparently accumulated a large number of deletions/duplications after they diverged. This was reflected by the fact that ten of the 65 probes used for comparative mapping detected different copy numbers between the two genomes and that there were several discrepancies in marker locations between the two species.

The significant level of homology observed here between mungbean and lablab has also allowed a detailed examination of homoeologous chromosomal regions. Twelve of the 13 linkage groups of mungbean were able to be compared to 17 of the lablab groups. In some cases, unlinked lablab groups were able to be tentatively joined on the basis of their common loci with mungbean, and vice versa (Fig. 2). This result will need to be examined further by analysing interspersed mungbean loci on the lablab map to determine if there is actual linkage between currently unlinked groups. Nonetheless, the results indicated that the 17 represented linkage groups in the current lablab map could be consolidated into 12 groups on the basis of their synteny with the mungbean groups, and the 13 mungbean groups into 12.

It is of interest to note that groups 2 and 13 of lablab and group I of mungbean appear to be involved in a complicated chromosomal rearrangement event. Markers LpCS328 and LpCS314 both mapped to group I of mungbean, but showed no linkage to any of the markers on group 2 of lablab as was expected by those surrounding markers common to both genomes. Instead these two probes map to group 13 of lablab (Fig. 2). This result indicated that linkage group 13 of lablab is very likely to represent the “extra” chromosome in this species compared to those of mungbean. It seems that the “extra” chromosome is unlikely to have been derived via partial genome duplication. Rather it was more likely derived by an event of chromosome division or integration. This speculation is supported by the fact that this type of phenomenon was not observed in any other genomic region.

The strategy of the comparative mapping of mungbean and lablab has shown to be very effective in increasing the coverage of both the current mungbean and lablab linkage maps, as well as increasing the efficacy of probes from both species. It has also allowed the identification of linkage groups (chromosomes) that were likely to be involved in the change of basic chromosome numbers between the two species. Furthermore, this study has identified regions of synteny between these relatively distant members of the Fabaceae and provided additional evidence for the existence of highly related genomes throughout the family. This, in turn, may facilitate more powerful identification of genes conditioning traits of interest in both of these species by the comparative mapping approach.

Acknowledgements The authors thank Dr. Nevin Young for his kind donation of the mungbean and cowpea clones used in the mungbean and lablab maps and thank Dr. Frank Smith for his critical reading of the manuscript. This project was partially funded by the Grains Research and Development Corporation (grant number CSC46). The experiments described in this manuscript comply with the current laws of Australia, where they were conducted.

References

Arumuganathan K, Earle ED (1991) Nulcear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Asnaghi C, Paulet F, Kaye C, Grivet L, Deu M, Glaszmann JC, D' Hont A (2000) Application of synteny across the Poaceae to determine the map location of a sugarcane rust resistance gene. Theor Appl Genet 101:962–969

Bonierbale MW, Plaisted RL, Tanksley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095–1104

Boutin SR, Young ND, Olson TC, Yu ZH, Shoemaker RC, Vallejos CE (1995) Genome conservation among three legume genera detected with DNA markers. Genome 38:928–937

Burr B, Burr FA, Thompson KH, Albertson MC, Stuber CW (1988) Gene mapping with recombinant inbreds in maize. Genetics 118:519–526

Concibido VC, Young ND, Lange DA, Denny RL, Danesh D, Orf JH (1996) Targeted comparative genome analysis and qualitative mapping of a major partial-resistance gene to the soybean cyst nematode. Theor Appl Genet 93:234–241

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: version II. Plant Mol Biol Rep 1:19–21

Devos KM, Gale MD (2000) Genome relationships: the grass model in current research. Plant Cell 12:637–646

Fatokun CA, Menancio-Hautea DI, Danesh D, Young ND (1992) Evidence for orthologous seed-weight genes in cowpea and mung bean based on RFLP mapping. Genetics 132:841–846

Gadish I, Zamir D (1987) Differential zygotic abortion in an interspecific *Lycopersicon* cross. Genome 29:156–159

Gale MD, Devos KM (1998) Plant comparative genetics after 10 years. Science 282:656–659

Helentjaris T (1993) Implications for conserved genomic structure among plant species. Proc Natl Acad Sci USA 90:8308–8309

Kaga A, Ishii T, Tsukimoto K, Tokoro E, Kamijima O (2000) Comparative molecular mapping in *Ceratotropis* species using an interspecific cross between azuki bean (*Vigna angularis*) and rice bean (*V. umbellata*). Theor Appl Genet 100:207–213

Konduri V, Godwin ID, Liu CJ (2000) Genetic mapping of the *Lablab purpureus* genome suggests the presence of a 'cuckoo' gene(s) in this species. Theor Appl Genet 100:866–871

Lagercrantz U, Lydiate DJ (1996) Comparative genome mapping in *Brassica*. Genetics 144:1903–1910

Lambrides CJ, Lawn RJ, Godwin ID, Manners J, Imrie BC (2000) Two genetic linkage maps of mungbean using RFLP and RAPD markers. Aust J Agric Res 51:415–425

Liu CJ, Witcombe JR, Pittaway TS, Nash M, Hash CT, Busso CS, Gale MD (1994) An RFLP-based genetic map of pearl millet (*Pennisetum glaucum*). Theor Appl Genet 89:481–487

Liu CJ, Devos KM, Witcombe JR, Pittaway TS, Gale MD (1996) The effect of genome and sex on recombination rates in *Pennisetum* species. Theor Appl Genet 93:902–908

Manly KF, Olson JM (1999) Overview of QTL mapping software and introduction to Map Manager QT. Mammal Genome 10:327–334

Maughan PJ, Saghai-Maroof MA, Buss GR (1996) Molecular-marker analysis of seed-weight: genomic locations, gene action, and evidence for orthologous evolution among three legume species. Theor Appl Genet 93:574–579

Menancio-Hautea D, Fatokun CA, Kumar L, Danesh D, Young ND (1993a) Comparative genome analysis of mungbean (*Vigna radiata* L. Wilczek) and cowpea (*Vigna unguiculata* L. Walpers) using RFLP mapping data. Theor Appl Genet 86:797–810

Menancio-Hautea D, Kumar L, Danesh D, Young N (1993b) A genome map for mungbean [*Vigna radiata* (L.) Wilczek] based on DNA genetic markers (2n = 2*x* = 22). In: O'Brien JS (ed) Genetic maps 1992. A compilation of linkage and restriction maps of genetically studied organisms. Cold Spring Harbor Laboratory Press, New York, pp 259–261

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Robertson DS (1984) Different frequency in the recovery of crossover products from male and female gametes of plants hypoploid for B-A translocations in maize. Genetics 107:117–130

Sharp PJ, Kreis M, Shewry PR, Gale MD (1988) Location of beta-amylase sequences in wheat and its relatives. Theor Appl Genet 75:286–290

Tanksley SD, Bernatzky R, Lapitan NL, Prince JP (1988) Conservation of gene repertoire but not gene order in pepper and tomato. Proc Natl Acad Sci USA 85:6419–6423

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, Young ND (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Vallejos CE, Sakiyama NS, Chase CD (1992) A molecular marker-based linkage map of *Phaseolus vulgaris* L. Genetics 131:733–740

Vicente MC de, Tanksley SD (1991) Genome-wide reduction in the recombination of backcross progeny derived from male versus female gametes in an interspecific cross of tomato. Theor Appl Genet 83:173–178

Weeden NF, Muehlbauer FJ, Ladizinsky G (1992) Extensive conservation of linkage relationships between pea and lentil genetic maps. J Hered 83:123–129