Theor Appl Genet (2000) 100:866–871

V. Konduri · I.D. Godwin · C.J. Liu

Genetic mapping of the *Lablab purpureus* genome suggests the presence of 'cuckoo' gene(s) in this species

Received: 20 April 1999 / Accepted: 23 August 1999

Abstract A linkage map of *Lablab purpureus* consisting of 127 RFLP and 91 RAPD loci was constructed in an F_2 population of 119 individuals. This population was derived from a cross between 'Rongai' (an annual cultivar) and CPI 24973 (a perennial wild accession). The map comprises 17 linkage groups and covers 1610 centiMorgans (cM) with an average distance of 7 cM between markers. Severe segregation distortions were observed, with the very extreme situation where no paternal type was recovered from the mapping population. These results strongly suggest the presence of a gene conferring preferential transmission from the maternal parent 'Rongai'. It was also clear that, while the majority of RAPD markers are valuable when used together with RFLP or other stringent marker systems, they could be problematic when used solely in mapping exercises.

Key words *Lablab purpureus* · Genetic markers · Linkage map · Preferential transmission · Segregation distortion

Introduction

Lablab purpureus (2n=24, Philip 1982) is a legume species grown in the tropics and the subtropics of the world. Commonly known as bonavist, Egyptian, hyacinth or lablab bean, it is believed to be a native of India, southeast Asia or Africa (Shivashanker et al. 1993). Tolerant to drought and high temperature, and able to adapt to a wide range of soils and climates, lablab is a popular vegetable legume in Asia. Lablab is also known to produce large quantities of green material with a high protein concentration, and is widely cultivated as forage and green manure (Kay 1979).

Lablab is extensively used as a ley legume in northern Australia. It provides a high yield of forage for beef cattle grazing and improves the yield and protein content of the subsequent grain crops (Lloyd et al. 1991). A lablab breeding program with the objective of selecting perennial forage cultivars is currently in progress in Queensland, Australia. To facilitate the breeding activities, we embarked on work to develop molecular markers, construct linkage maps, and tag genes of agronomic importance. This paper is the first report on the construction of a linkage map in *L. purpureus* and provides evidence for the existence of a gene(s) conferring preferential transmission in this species.

Communicated by P. Langridge

V. Konduri · I.D. Godwin
School of Land and Food, University of Queensland, St. Lucia, Qld 4072, Australia

V. Konduri · C.J. Liu (✉)
CSIRO Tropical Agriculture, 306 Carmody Road, St. Lucia, Qld 4067, Australia
e-mail: Chunji.Liu@tag.csiro.au
Tel.: +61-7-3214 2223, Fax: +61-7-3214 2288

Materials and methods

Plant material

Based on a previous diversity study (Liu 1996), the annual cultivar 'Rongai' and the wild accession CPI 24973 were chosen as parents for the mapping population. The artificial hybridization between the two parents was carried out in a glasshouse. F_1 hybrids were selfed to produce F_2 populations. One of these populations, consisting of 119 individuals, was used in this study.

DNA extraction

DNA isolation from the plants was performed following the protocol modified from Sharp et al. (1988). Twenty milliliters of extraction buffer (100°mM Tris-HCl pH 8.5, 100 mM NaCl, 50 mM EDTA pH 8.0, and 2% SDS) were added to 0.5 g of freeze-dried leaf tissue and incubated for 1–2 h at 65°C. The contents were then extracted once with an equal volume of phenol/chloroform and once with chloroform. DNA was precipitated with ethanol and dissolved in 5 ml of 1×TE buffer. RNA was removed by digesting with RNAse and this was followed by phenol/chloroform- and chloroform-extractions. Purified DNA was then dissolved in 1×TE buffer.

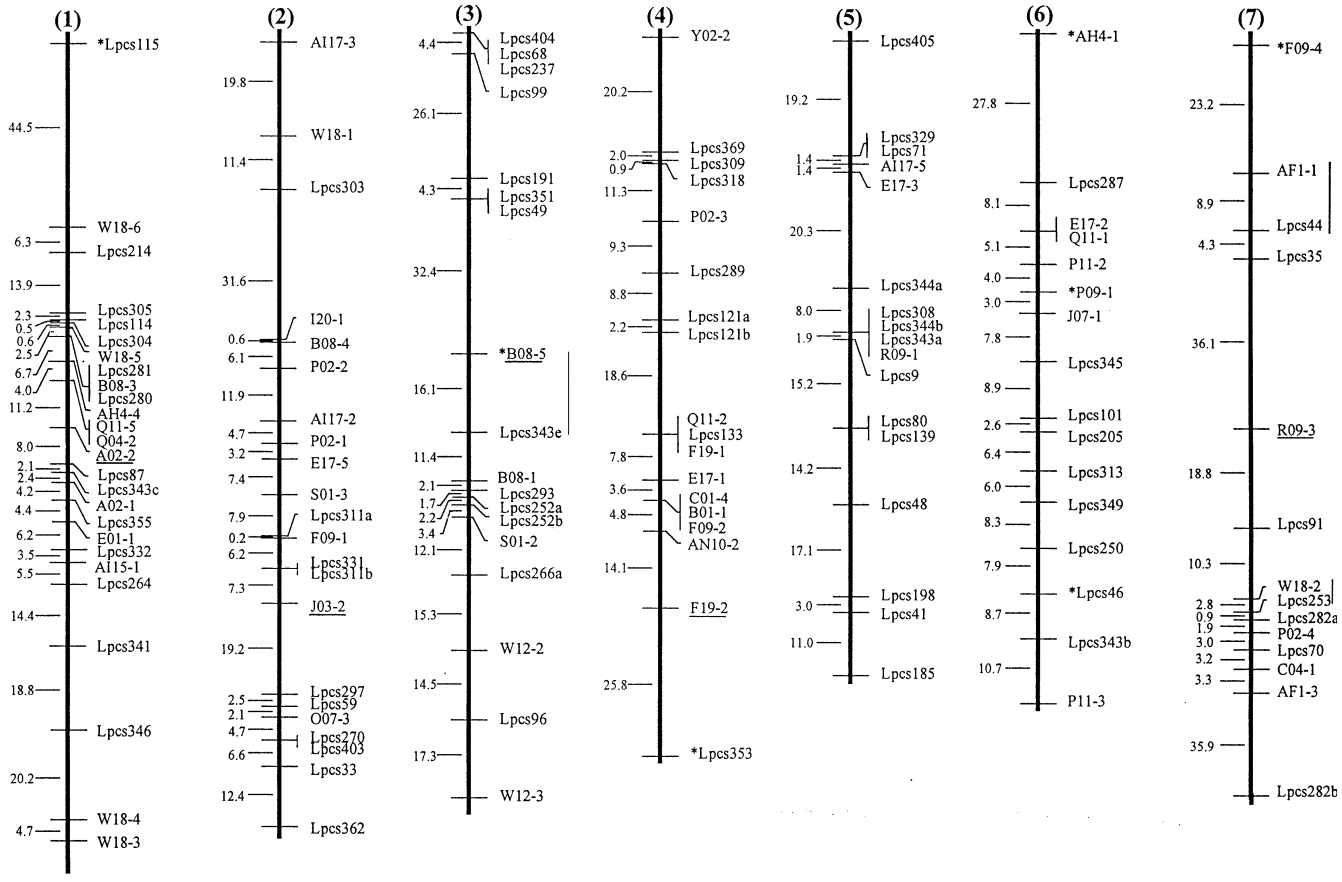

Fig. 1 The linkage map of *L. purpureus*. Linkage groups are ordered based on the number of loci and the genetic length. *Numbers* to the left of the vertical bars indicate the distances in cM, and locus names are listed to the right of the bars. RFLP loci are named as Lpcs followed by the corresponding probe numbers. Loci detected by the same probe are denoted with an extension, a, b, c, etc. RAPD loci are named after their respective primers followed by a series number indicating the fragment scored. Loci showing segregation distortions are indicated with an *asterix* (*). Ambiguous orders are indicated with a *vertical line* on the right side of the loci involved

RFLP probes

All RFLP probes used in this study were *Pst*I clones constructed using total genomic DNA from leaves of *L. purpureus* cv 'Rongai'. These clones were designated as LpCS1–LpCS550, where 'Lp' represented the species name *L. purpureus*, and 'CS', CSIRO, the organization where the work was carried out. The method used for obtaining these clones was that described by Liu et al. (1994) with the modification that pBluescript II SK^+ was used as the cloning vector.

Inserts from these clones were amplified using M13 forward and reverse primers. The PCR-amplified inserts were screened for the presence of highly repetitive sequences by the dot-blot technique. The inserts were probed with ^{32}P-labelled total genomic DNA digested with *Hae*III; those giving a positive signal were believed to be highly repetitive sequences and hence were discarded.

RFLP procedure

Restriction enzyme digestion, gel electrophoresis, Southern transfer, probe labelling and filter hybridizations were as described by Sharp et al. (1988), with the exception that Hybond N^+ nylon membrane (Amersham) was employed. For parental screening, six enzymes were used to begin with (*Eco*RI, *Eco*RV, *Dra*I, *Hind*III, *Hae*III, *Bam*HI), and this was subsequently reduced to three (*Eco*RI, *Eco*RV and *Hae*III). The membranes were washed three times at 65°C for 15 min each, once with 2×SSC and 1% SDS, then with 1×SSC and 1% SDS, and a final wash with 0.2×SSC and 0.5% SDS. Membranes were stripped for re-probing in a boiling solution of 0.5% SDS and 0.1×SSC for 15–20 min.

RAPD procedure

RAPD analyses were performed using random decamer primers obtained from Operon Technologies Inc. The polymerase chain reaction (PCR) was carried out using the method described by Liu (1996). The amplification products were separated on 1.5% agarose gels in 0.5% TBE buffer and visualized by staining with ethidium bromide. RAPD loci are named after their respective primers followed by a number which represents the DNA fragment scored.

Data analyses

All RAPD fragments were scored as dominant markers and most RFLP fragments were scored as co-dominant markers. Linkage analysis was carried out using the computer package MAPMAKER version 3.0 (Lander et al. 1987). Linkage groups were established at LOD≥4.0 and a maximum distance of 30 cM. Multipoint analysis was used to determine the relative order of markers in each group at LOD≥3.0. Loci showing deviations from the expected Mendelian segregation ratios of 1:2:1 (co-dominant) and 3:1 (dominant) were identified using the chi-square test.

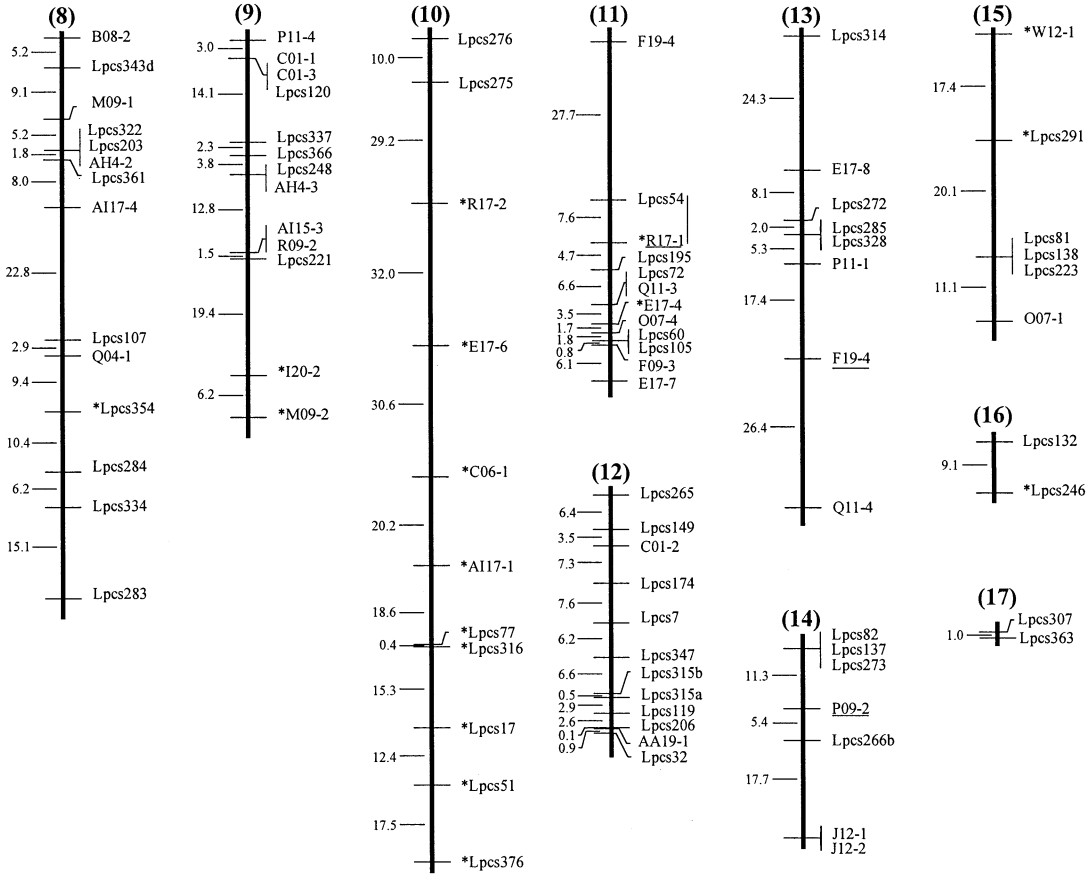

Fig. 1 (continued)

Results

*Pst*I genomic clones

Of the 550 putative recombinant clones selected, 407 gave PCR-amplification products. Seven percent of these 407 inserts produced strong signals when hybridised with *Hae*III-digested total genomic DNA, indicating that they were likely to detect high-copy sequences and hence were discarded. Of the remaining inserts, 85% detected single fragments as evident from hybridisation patterns with three restriction enzymes. Five percent detected two fragments, and the remaining 10% detected three or more fragments.

Polymorphisms

Initial parental screening with 20 RFLP probes revealed that, of the six restriction enzymes used, the majority of RFLPs could be detected by three of them, *Eco*RI, *Eco*RV and *Hae*III. Thus the other three restriction enzymes, *Dra*I, *Hind*III and *Bam*H1, were removed from further screening. Of the 324 single- or low (2–5)-copy RFLP probes screened, 168 were found to be polymorphic (51.85%) with the three restriction enzymes.

Similarly, RAPD markers also showed a high level of polymorphism between the two parents. Out of the 60 primers screened 35 (58.33%) generated clear polymorphic fragments between the two parents. Out of a total 210 RAPD fragments, 102 were polymorphic (48.5%), which corresponded to 2.93 polymorphic fragments per primer.

The linkage map

One hundred and sixteen of the polymorphic probes were used for mapping. These probes detected 127 loci, 109 of which were scored as co-dominant and the other 18 (14%) as dominant.

All the RFLP markers and 91 of the 102 RAPD markers were included in these linkage groups. Two hundred and eighteen markers formed 17 linkage groups (Fig. 1). Each of the remaining 11 RAPD fragments (AF1–2, AI15–2, AN10–1, C6–2, F19–3, J3–1, J7–2, O7–2, R17–3, S1–1 and Y2–1), although showing clear segregation (Fig. 2), was not linked to any of the other markers. The average genetic distance between RFLP/RFLP markers (6.1 cM) was significantly shorter than that between RAPD/RAPD or between RAPD/RFLP markers (9.1 cM). The 17 linkage groups cover a total genetic

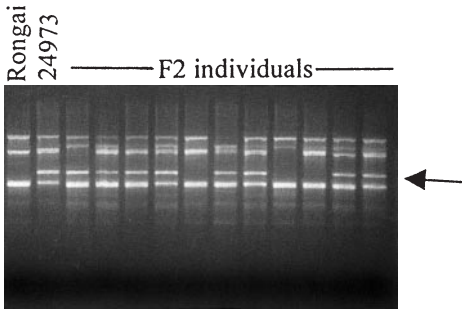

Fig. 2 The segregation pattern of an unlinked RAPD fragment (marked by an *arrow*) detected by primer Y02

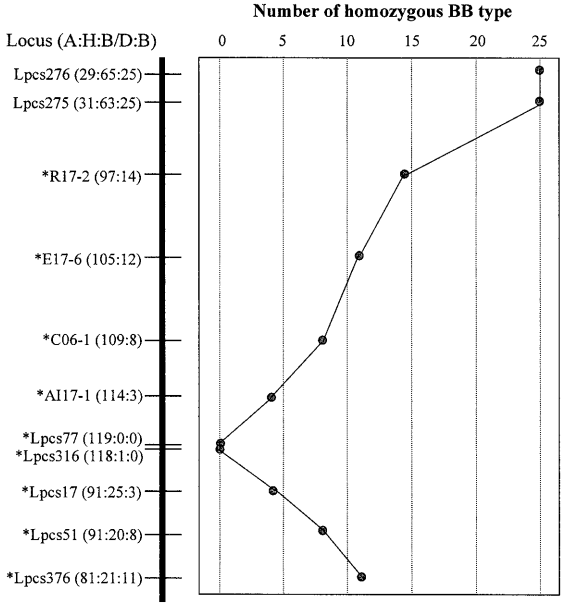

Fig. 3 The number of homozygous paternal BB types detected by markers on linkage group 10. For co-dominant markers the number of individuals showing homozygous maternal type of segregation are indicated as (*A*) while heterozygous types are indicated as (*H*) and homozygous paternal types as (*B*). For dominant markers, homozygous maternal or heterozygous types are indicated as (*D*) and homozygous paternal types as (*B*). Distorted loci are indicated with an asterix (*)

distance of 1610 cM with an average distance of 7 cM between loci. In several instances, inclusion of a RAPD marker dramatically increased the apparent genetic distances between two RFLP markers (Fig. 1). For example, the distance between Lpcs49 and Lpcs343e (32.5 cM) in linkage group 3 was increased by 16.0 cM (about 50%) due to the inclusion of the RAPD marker B08–5.

Duplicated RFLP loci

Of 116 RFLP probes used, seven hybridized to sequences that mapped to two loci each and another one mapped to five loci. These duplicated loci did not form clusters and appeared to disperse randomly across the genome (Table 1). These markers provided no evidence to suggest that large duplicated segments were present in this genome.

Table 1 Distribution of duplicated RFLP loci

Probe	Number of loci detected	Location (linkage group)
Lpcs121	2	4, 4
Lpcs252	2	3, 3
Lpcs266	2	3, 14
Lpcs282	2	7, 7
Lpcs311	2	2, 2
Lpcs315	2	12, 12
Lpcs344	2	5, 5
Lpcs343	5	1, 3, 5, 6, 8

Segregation distortion

RFLP and RAPD markers that showed deviations from the expected 1:2:1 or 3:1 segregation ratios were observed in 10 of the 17 linkage groups (Fig. 1). The most severely distorted loci were clustered on linkage group 10. One of these markers, Lpcs77, located in the middle of the group, detected only the homozygous female parental type (AA). No allele from the male parent CPI 24973, either as a homozygous (BB) or heterozygous (AB) type, was present at this locus. The marker Lpcs316, closely linked to Lpcs77, also detected no homozygous BB type. But unlike Lpcs77, Lpcs316 did identify one heterozygous AB type from the 119 F_2 individuals. The severity of distortion for markers on both sides of these two markers progressively waned (Fig. 3) with markers at one end of the linkage group conforming perfectly to the expected segregation ratio.

Discussion

The linkage map

The linkage map reported here consists of 17 linkage groups although the haploid chromosome number of lablab is only 12 (Philip 1982). This suggested that a proportion of the lablab genome has not been covered by the existing markers. However, a ‘perfect map’ (i.e. with the number of linkage groups equal to the number of haploid chromosomes) of the lablab genome does not necessarily mean that the total map distance of 1610 cM would be dramatically increased. Genetic distances between some of the markers in the current map might have been overestimated due to the inclusion of a large number of RAPD markers. It seems evident that some RAPD markers, although appearing to have a clear segregation (Fig. 2), could not be scored properly. Evidence for this includes: (1) 11 of the 102 RAPD markers showed no linkage between themselves or to any other marker, while all the 127 RFLP markers were mapped; (2) inclu-

sion of a RAPD marker dramatically increased genetic distances between some RFLP markers (Fig. 1), and (3) the average genetic distance between RAPD/RAPD or RAPD/RFLP markers (9.1 cM) was significantly longer than that between RFLP markers (6.1 cM).

As noted in previous studies, this difficulty in scoring RAPD fragments could be due to the sensitivity of the system and/or competition between different priming sites (Kennard et al. 1994; Halldén et al. 1996). These errors in classifying RAPD fragments would inevitably lead to exaggerated estimates of genetic distances and an increase in the number of unlinked markers.

Segregation distortion and the presence of a putative 'cuckoo' gene(s)

Irrespective of the marker systems employed, deviation from the expected Mendelian segregation ratios of 1:2:1 or 3:1 is common in mapping exercises. Segregation distortion may be due to gametophytic competition or sporophytic selection (O'Donoughue et al. 1992), and the severity of distortion is affected by sex and by parental interactions (Liu et al. 1996). Distorted loci are present in genetic maps of almost all species studied so far. However, to our knowledge, there is no report of the extreme distortion that has been observed in this study. Two loci in this study detected no homozygous paternal (BB) type (Fig. 3). One of these, Lpcs77, detected only the homozygous female parental (AA) type. Although polymorphic between the two parents, the probe could either be a fragment from cytoplasmic DNA, or be monomorphic between the gametes that formed the mapping population. Unfortunately, DNA from the hybrid plant was not available for testing these possibilities. However, Lpcs316, the probe closely related to Lpcs77, did detect a single heterozygous AB type, indicating that the location of the latter is likely to be genuine.

As indicated in other studies (Busso et al. 1995; Liu et al. 1996), the strong deviations against AB or BB types seemed to suggest that the paternal parent (BB) might carry a lethal gametocidal gene in this chromosomal region. This 'lethal' gene only becomes lethal in combination with cytoplasm from the female parent 'Rongai'. Otherwise it would be difficult to explain how the male parent (homozygous for this locus) could exist and how it could successfully produce fertile hybrids (AB) with 'Rongai' (from which the mapping population was derived).

In fact, the behaviour of this 'lethal' gene(s) is similar to that of the 'cuckoo' chromosomes identified in several grass species (Mann 1975; Endo and Katayama 1978; Miller et al. 1982). In wheat, such 'cuckoo' chromosomes have been identified which in a hetero- or hemizygous condition kill gametes lacking them (King et al. 1991a). This ensures that all gametes lacking the 'cuckoo' genes are non-functional, and therefore those gametes containing the genes are preferentially transmitted. This is exactly what we observed in the present study with the exception that the 'cuckoo' gene is not from the wild but rather from the cultivated parent. The location of such a 'cuckoo' gene conferring preferential transmission in this chromosome region is supported by the fact that, when moving away from this region, the severity of distortion for markers on both sides progressively waned (Fig. 3).

'Cuckoo' chromosomes have been exploited in developing new crop cultivars (e.g. King et al. 1991a, b). However, all 'cuckoo' chromosomes identified so far are derived from wild species (Miller et al. 1982; King et al. 1992) and these chromosomes have difficulty in recombining with their counterparts in cultivated crops. This difficulty has limited the application of 'cuckoo' chromosomes to cases involving centric breakage and fusion (Robertsonian translocations) (King et al. 1992). The rate of success in applying these translocations in variety development, however, can be low due to the presence of deleterious genes on the alien chromatin (Knott 1980; King et al. 1992). The way to circumvent this difficulty is to use only a small chromosome fragment containing the 'cuckoo' gene. The relatively high rate of recombination around the 'cuckoo' locus observed in the present study indicates that it should be possible to isolate the gene using a map-based cloning approach. The molecular markers linked to the locus and the availability of a bacterial artificial chromosome library in lablab (Liu et al. 1998) should facilitate such an endeavor.

Acknowledgments We are grateful to Ms. Cristine Cox for helping to maintain the mapping population. This project was funded by the Grains Research and Development Corporation (grant No. CS28 N) to C.J.L., and V.K is supported by an AusAID scholarship. The experiments comply with the current laws of Australia.

References

Busso CS, Liu CJ, Hash CT, Witcombe JR, Devos KM, de Wet JMJ, Gale MD (1995) Analysis of recombination rate during male and female gametogenesis in pearl millet (*Pennisetum glaucum*) using RFLP markers. Theor Appl Genet 90:242–246

Endo TR, Katayama Y (1978) Finding of a selectively retained chromosome of *Aegilops caudata* L. in common wheat. Wheat Inf Serv 47–48, 32–35

Halldén C, Hansen M, Nilsson NO, Hjerdin A, Säll T (1996) Competition as a source of errors in RAPD analysis. Theor Appl Genet 93: 1185–1192.

Kay D.E. (1979) Crop and product digest no.3, food legumes. Tropical Products Institute, London

Kennard WC, Poetter K, Dijkhuizen A, Meglic V, Staub JE, Havey MJ (1994) Linkages among RFLP, RAPD, isozyme, disease-resistant and morphological markers in narrow and wide crosses of cucumber. Theor Appl Genet 89:42–48

King IP, Koebner RMD, Reader SM, Miller TE (1991a) Induction in the male fertility gene of the preferentially transmitted *Aegilops sharonensis* chromosome $4S^l$ and its application for hybrid wheat production. Euphytica 54:33–39

King IP, Koebner RMD, Schlegel R, Reader SM, Miller TE, Law CN (1991b) Exploitation of a preferentially transmitted chromosome from *Aegilops sharonensis* for the elimination of segregation for height in semi-dwarf bread wheat varieties. Genome 34:944–949

King IP, Purdie KA, Miller TE, Law CN, Rogers WJ (1992) Exploitation of chromosome $4S^l$, from *Aegilops sharonensis*,

for the production of stable 44-chromosome wheat lines. Heredity 69:160–165
Knott DR (1980) Mutation of a gene for yellow pigment linked to *Lr19* in wheat. Can J Genet 22:651–654
Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181
Liu CJ (1996) Genetic diversity and relationships among *Lablab purpureus* genotypes evaluated using RAPDs as markers. Euphytica 90:115–119
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
Liu CJ, Tao QZ, Zhang HB (1998) Construction of a bacterial artificial chromosome library of *Lablab purpureus* in a plant transformation vector In: Larkin PJ (ed) Proc 4th Asia-Pacific Conf on Agricultural Biotechnology, Darwin, Australia, pp 177–178
Lloyd DL, Smith KP, Clarkson NM, Weston EJ, Johnson B (1991) Sustaining multiple production systems. 3. Ley pastures in the subtropics. Trop Grassl 25:181–188
Mann SS (1975) Exclusive preferential transmission of an alien chromosome in common wheat. Crop Sci 16:580–583
Miller TE, Hutchinson J, Chapman V (1982) Investigation of a preferentially transmitted *Aegilops sharonenesis* chromosome in wheat. Theor Appl Genet 61:27–33
O'Donoughue LS, Wang Z, Röder M, Kneen B, Leggett M, Sorrells ME, Tanksley SD (1992) An RFLP-based linkage map of oats based on a cross between two diploid taxa (*Avena atlantica*×*A. hirtula*). Genome 35:765–771
Philip T (1982) Induced tetraploidy in *Dolichos lablab* Linn. Curr Sci 51:945
Sharp PJ, Kreis M, Shewry PR, Gale MD (1988) Location of β-amylase sequences in wheat and its relatives. Theor Appl Genet 75:286–290
Shivashankar G, Kulkarni RS, Shashidhar HE, Mahishi DM (1993) Improvement of field bean. In: Chadha KL, Kallo G (eds) Advances in horticulture. Vol 5, vegetable crops. New Delhi, pp 277–286