Development of a sequence-specific PCR marker linked to the gene "*pauper*" conferring low-alkaloids in white lupin (*Lupinus albus* L.) for marker assisted selection

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Abstract Seeds and plants of wild type *Lupinus albus* are bitter and contain high level of alkaloids. During domestication, at least three genes conferring low-alkaloid content were identified and incorporated into commercial varieties. Australian lupin breeders exclusively utilize one of these sweetness genes, "*pauper*", in all varieties to prevent possible bitterness contamination via out-crossing. A cross was made between a sweet variety Kiev Mutant (containing *pauper* gene) and a bitter type landrace P27174, and the population was advanced into F_8 recombinant inbred lines (RILs). Twenty-four plants representing sweetness and bitterness were subjected to DNA fingerprinting by the microsatellite-anchored fragment length polymorphism (MFLP) technique. A dominant polymorphism was discovered in an MFLP fingerprint. The MFLP marker was converted into a co-dominant, sequence-specific, simple PCR-based marker. Linkage analysis by the software program MapManager with marker score data and alkaloid phenotyping data from a segregating population containing 190 F_8 RILs indicated that the marker is linked to the *pauper* gene at the genetic distance of 1.4 centi-Morgans (cM). This marker, which is designated as "PauperM1", is capable of distinguishing the *pauper* gene from the other two low-alkaloid genes *exiguus* and *nutricius*. Validation on germplasm from the Australian lupin breeding program showed that the banding pattern of the marker PauperM1 is consistent with the alkaloid genotyping on a wide range of domesticated varieties and breeding lines. The PauperM1 marker is now being implemented for marker assisted selection in the Australian albus lupin breeding program.

Keywords Marker assisted selection (MAS) ·
Molecular marker · MFLP · *Lupinus albus*

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Introduction

White (or albus) lupin (*Lupinus albus* L.) has been cultivated for over 3,000 years in the Mediterranean region (Gladstones 1970). All wild-types and traditional landraces are "bitter" with 1.5–2% alkaloid content in the seeds (Harrison and Williams 1982; Cowling et al. 1998). During domestication in the early twentieth century (Hondelmann 1984), several different "sweet" genes conferring low-alkaloids in *L. albus* were discovered (Kurlovich 2002). Three of the genes, *pauper*, *exiguus*, and *nutricius*, were incorporated into commercial varieties (Harrison and Williams 1982). Genetic analysis confirmed that these three low-alkaloid genes are inherited independently (Harrison and Williams 1982). The alkaloid chemical components controlled by each of the three genes are different; but plants containing the *pauper* gene have the lowest total alkaloid content (Harrison and Williams 1983), typically 20 mg total alkaloids kg^{-1}, well below the Australian and New Zealand Food Standard for lupins of 200 mg kg^{-1}. Most modern varieties of *L. albus* are "sweet" so that they can be fed to animals or used in human foods without risk of alkaloid poisoning. Bitter types are grown in some circumstances but they must be "de-bittered" by soaking the seeds in river or running water for two or three days before consumption. Sweet albus types are currently cultivated in Europe, countries of the former Soviet Union, South Africa, and North and South America. However, not all breeders in these countries employ the same gene to condition low-alkaloid. Sweet white lupin was introduced to Australia in the 1970s, and is now cultivated on sandy soils in Western Australia and on more-fertile, loamy soils in New South Wales in Australia.

Although white lupin is primarily a self-pollinated species, its insect-mediated out-crossing rate of 5–10% (Faluyi and Williams 1981; Huyghe 1997) is sufficiently high to risk contamination if there is more than one sweet gene in adjacent plant populations. When two plants with different low-alkaloid genes are crossed, the resultant F_1 is bitter (Harrison and Williams 1982). The bitterness is inherited, and the frequency of bitter contamination in a field crop increases by further out-crossing in future generations (Workman and Allard 1962). Bitter plants may also exhibit higher fitness and produce relatively higher seed numbers (Cowling et al. 1998). To avoid a

potential bitter seed contamination problem in the albus crop, Australian albus lupin breeders have agreed that all commercial varieties released in this country should exclusively utilize the *pauper* gene for maintaining low-alkaloid. In countries where bitter and sweet crops are grown in the same regions it is very difficult to maintain pure-breeding, low-alkaloid varieties.

Plant breeding of modern white lupin has a relatively short history, and the gene pool of domesticated white lupin with the *pauper* gene is limited. In white lupin breeding, bitter wild-types, and landraces, as well as domesticated lines with other low-alkaloid genes, are used in crossing to broaden the genetic base of the program. For the selection of sweet progeny from bitter × sweet crosses, the most widely used alkaloid detection method is to squeeze a drop of petiole sap onto a filter paper impregnated with Dragendorff reagents (Harrison and Williams 1982). The alkaloids in the sap from a bitter plant produce a reddish-brown color change, but sweet plants do not (Harrison and Williams 1982). A similar reaction occurs with saturated iodine solution. Exudates from soaked whole seeds can be tested non-destructively, or a cut seed surface can be dipped in iodine and the color change observed in situ. Bitter plants give rise to seeds with seed coats that appear as pink fluoresce under UV light of wavelength 365 nm (von Baer and Perez 1991). This is a quick and useful technique for estimating bitter seed frequency. However, the UV technique cannot detect heterozygotes which will produce a bitter plant (full alkaloid expression occurs about six weeks after germination).

Gas chromatography is capable of accurately determining and quantifying the precise alkaloid content in lupins (Harrison and Williams 1983). However, at present it remains a great challenge for lupin breeders to exclusively select progenies possessing the *pauper* gene because none of the simple, quick, inexpensive tests (Dragendorff, iodine, or UV) is capable of differentiating sweet plants with different low-alkaloid genes, or plants where more than one sweet gene is present.

A molecular marker for practical marker assisted selection (MAS) needs to be closely linked to a gene of interest, be cost-effective, be amenable to large numbers of samples, (Gupta et al. 1999), and be applicable to wide ranges of crosses in a breeding program (Yang et al. 2008). Similar to the DNA

fingerprinting method "Selectively amplified micro-satellite polymorphic loci" (SAMPL) described by Witsenboer et al. (1997), the "Microsatellite-anchored fragment length polymorphisms" (MFLP) technique targets microsatellite-related DNA polymorphisms by combining a SSR-anchor primer with an AFLP primer in the PCR (Yang et al. 2001). The advantages of MFLP are: (1) it is highly efficient in generating large numbers of DNA polymorphisms; and (2) many MFLP markers can easily be converted into sequence-spe-cific, simple PCR-based markers desirable for routine marker implementation (Yang et al. 2001, 2004). The objective of this study was to apply the MFLP technique to develop a molecular marker linked to the *pauper* gene for MAS in white lupin breeding.

Materials and methods

Plant materials for marker development

A single plant of a landrace from Ethiopia, P27174 (bitter), was used as a pollen donor and crossed onto a single plant of cv. Kiev Mutant (low-alkaloid contain-ing the *pauper* gene). Some of the resulting F_1 seeds were grown in a screenhouse to produce F_2 seeds. The progeny was advanced to F_8 recombinant inbred lines (RILs) by single seed decent with no bias. The parents, F_1, and 190 RILs were sown in a 1 m row in a screenhouse. When the plants were at the flowering stage on the primary inflorescence, a leaf was taken from the plant, and a drop of sap was squeezed from the petiole, which was dipped onto a dried filter paper treated with Dragendorff reagents (Harrison and Williams 1982). The presence of a brown-red pig-mentation showed the high content of alkaloids, and the absence of the pigmentation indicated low-alkaloid content. Each line was tested at least twice to ensure accuracy of the phenotyping.

Search for a candidate marker linked to the *pauper* gene by MFLP

Twenty-four plants, including the low-alkaloid parent Kiev Mutant (containing the gene *pauper*), the landrace P27174 (bitter), 11 sweet F_8 RILs, and 11 bitter F_8 RILs, were used in search for a candidate marker linked to the *pauper* gene. Fifteen seeds of each line were sown in a 25 cm-diameter pot filled

Table 1 Sequences of 10 SSR-anchor primers used in MFLP fingerprinting for generating candidate molecular markers linked to the gene *pauper* conferring low-alkaloids in *L. albus* L

Primer name	Sequence (5′–3′)
MF01	GTCCGAGAGAGAGAGA
MF02	GGCATGTGTGTGTGTG
MF11	GGACCTCTCTCTCTCT
MF21	CCCAAGAGAGAGAGAGAG
MF23	GGGCAGAGAGAGAGAG
MF42	GTCTAACAACAACAACAAC
MF43	CCTCAAGAAGAAGAAGAAG
MF51	GGGAACAACAACAAC
MF128	DVDTCTCTCTCTCTCTC[a]
MF129	HVHTGTGTGTGTGTGTG[b]

[a] D = A + G + T; V = A + G + C

[b] H = A + C + T

with river sand and maintained in a $20 \pm 2°C$ glasshouse. Two weeks after sowing, two leafs from each plants were taken, and the bulked 30 leaves for each line were ground into powder in liquid nitrogen. Genomic DNA was extracted using the method of Raeder and Broda (1985). Method of MFLP finger-printing was described by Yang et al. (2001). A total of 160 sets of MFLP fingerprints were obtained, which were produced by the 10 SSR-anchor primers listed in Table 1 each in combination with the 16 AFLP primers (*Mse*I-ANN) (Vos et al. 1995). The MFLP products were resolved on sequencing gels (5% acrylamide, 7 M urea) using a Sequi-Gen GT sequencing cell (Bio-Rad). Each gel had 96 samples comprising of four sets of MFLPs. The 24 plants in each set of MFLPs were arranged in the order of 4 bitter, 4 sweet intervals (Fig. 1). An MFLP polymor-phism is considered as a candidate marker linked to the *pauper* gene if its banding pattern matched with the alkaloid phenotyping on the 24 testing plants (Yang et al. 2002, 2004, 2008).

Conversion of the candidate MFLP marker into a sequence-specific PCR marker

DNA fragments from the candidate MFLP marker were isolated from the dried MFLP gel, re-amplified in PCR, cloned, and sequenced using standard proce-dures (Yang et al. 2004). To enable greater choice in primer design, the DNA sequence of the candidate
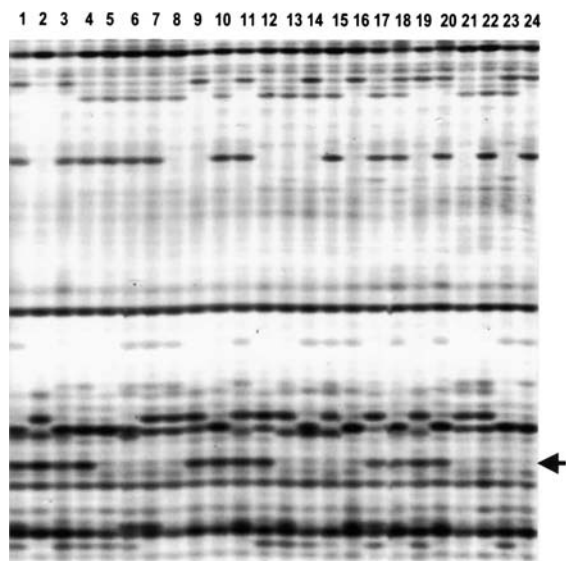
Fig. 1 Discovery of a candidate molecular marker linked to the *pauper* gene in *L. albus* on a MFLP fingerprint generated by SSR-anchor primer MF129 in combination with AFLP primer *Mse*I-AGT. The 24 tested plants were from a segregating population of F_8 derived recombinant inbred lines from a cross between Kiev Mutant (containing the *pauper* gene) and P27174 (bitter). Twelve testing plants were bitter, including the parent P27174 (Lane 1), RIL162 (Lane 2), RIL167 (Lane 3), RIL178 (Lane 4), RIL25 (Lane 9), RIL137 (Lane 10), RIL152 (Lane 11), RIL165 (Lane 12), RIL13 (Lane 17), RIL76 (Lane 18), RIL97 (Lane 19), RIL136 (Lane 20). The other 12 tested plants were sweet, including RIL7 (Lane 5), RIL68 (Lane 6), RIL72 (Lane 7), RIL89 (Lane 8), RIL3 (Lane 13), RIL61 (Lane 14), RIL87 (Lane 15), RIL100 (Lane 16), the sweet parent Kiev Mutant (Lane 21), RIL102 (Lane 22), RIL153 (Lane 23), and RIL195 (Lane 24). Arrow indicates the candidate MFLP marker (112 bp in length) linked to the bitter-allele of the *pauper* gene

marker band was extended beyond the SSR-end of the *Mse*I-SSR fragment obtained in the MFLP (Yang et al. 2001). This was achieved by digesting genomic DNA with restriction enzyme *Taq*I (recognition 5′-T/CGA-3′, Fermentas Life Sciences), followed by ligation of the restriction fragments with a modified *Mse*I-adaptor (Vos et al. 1995) but fitting the *Taq*I restriction sites (one string is 5′-GACGATGAGTCCTGAG-3′, and the other string is 5′-CGCTCAGGACTCAT-3′). A PCR was conducted using one sequence-specific primer designed from the existing sequence of the MFLP fragment, in combination with the *Taq*I-adaptor primer (5′-GACGATGAGTCCTGAGCGA-3′). Details of this DNA extension method were described by You et al. (2005).

A pair of sequence-specific primers was designed, based on the DNA sequence of the candidate marker, so that the annealing temperature was approximately 54°C calculated using the nearest-neighbor model (http://row.sigma-genosys.eu.com). Screening for the converted marker was performed by PCR using primer "PauperM1F" (5′-AAGAAAAGGCCCAATG-3′; labeled with γ-^{33}P) and "PauperM1R" (5′-TTTTAAA GTCATACCATTGAG-3′) with un-digested genomic DNA as templates. A PCR was cycled on a Hybaid DNA Express thermocycler for 30 cycles each of 94°C for 30 s, 54°C for 30 s, and 72°C for 1 min. The amplified products were resolved on a sequencing gel (as described above) at 55 W for 2 h, and were detected by autoradiography using an X-ray film (Yang et al. 2001, 2004, 2008).

Linkage confirmation between the marker and the *pauper* gene

The converted marker was tested on the segregating population of 190 F_8 RILs derived from the cross Kiev Mutant × P27174. The marker score data and the Dragendorff testing phenotyping data were merged and analyzed by the software program MapManager (Manly et al. 2001) to determine the genetic linkage between the marker and the *pauper* gene.

Validation of converted marker on commercial varieties

The established molecular marker was tested on eight reference sweet varieties of *L. albus* representing the three low-alkaloid genes *pauper*, *exiguus*, and *nutricius* (Gladstones 1970; Harrison and Williams 1982). The marker was also tested on 13 randomly selected bitter landraces of *L. albus*. The reference sweet varieties and the bitter landraces were sourced from the Lupin Germplasm Collection at the Department of Agriculture and Food Western Australia. Their origins and alkaloid phenotypes are listed in Table 2.

Sweet germplasm containing 185 lines from the Australian lupin breeding program, including local and foreign varieties, and breeder's lines, were also employed to validate the correlation between the marker banding pattern and the alkaloid genotypes. The tested lupin accessions included 185 sweet lines with *puaper* gene, 24 bitter lines, and 7 "non-*pauper*"

Table 2 Validation of sequence-specific PCR marker "PauperM1" on varieties and landraces of *L. albus* L representing various alkaloid genotypes

Lines	Origin	Alkaloid genotype	"PauperM1" marker score[a]
Kiev Mutant	Ukraine	Sweet, *pauper*	PauperM1[S]
Kraffquell	Germany	Sweet, *pauper*	PauperM1[S]
Pflugs Gela	Germany	Sweet, *pauper*	PauperM1[S]
Ultra	Germany	Sweet, *pauper*	PauperM1[S]
Astra	Chile	Sweet, *pauper*	PauperM1[S]
Neuland	Germany	Sweet, *exiguus*	PauperM1[B]
Start	Russia	Sweet, *exiguus*	PauperM1[B]
Nahrquell	Germany	Sweet, *nutricius*	PauperM1[B]
P27174[b]	Ethiopia	Bitter	PauperM1[B]
P24731	Greece	Bitter	PauperM1[B]
P25769	Greece	Bitter	PauperM1[S, c]
P25785	Greece	Bitter	PauperM1[B]
P25787	Greece	Bitter	PauperM1[S, c]
P27499	Portugal	Bitter	PauperM1[B]
P27556	Portugal	Bitter	PauperM1[B]
P27670	Turkey	Bitter	PauperM1[B]
P27714	Egypt	Bitter	PauperM1[B]
P28551	Ethiopia	Bitter	PauperM1[B]
P25758	Greece	Bitter	PauperM1[B]
P26730	Australia	Bitter	PauperM1[B]
P27641	France	Bitter	PauperM1[B]

[a] PauperM1[B] = bitter-allele marker band; PauperM1[S] = sweet-allele marker band

[b] P number is accession number in the Perth Lupin Germplasm Collection

[c] Two wild genotypes from Greece which showed a broken gene-marker linkage

sweet lines, which were all from New South Wales Department of Primary Industries (NSWDPI), Wagga Wagga, Australia. Sweet genotypes with an unknown low-alkaloid gene were crossed with Kiev Mutant (*pauper*). A minimum of eight individual F_1 plants were tested using the Dragendorff method (Harrison and Williams 1982). If the resultant F_1 plants were bitter, then complementation had occurred and the parent genotype was designated as "non-*pauper*" (either *exiguus* or *nutricius*).

Results

Phenotyping of alkaloids on the segregating marker population

Dragendorff tests showed that the landrace P27174 and the F_1 seeds were all bitter, while parent Kiev Mutant was sweet. Of the 190 F_8 RILs derived from the cross Kiev Mutant × P27174, 96 lines were identified as bitter, while the other 94 lines were sweet. The segregation of bitter : sweet in the F_8 fits

the expected 1:1 ratio ($\chi^2 = 0.244$, $P = 0.885$), confirming that the low-alkaloid character was controlled by a single recessive gene, which is consistent with the original description of this *pauper* gene (Gladstones 1970).

Marker development

From the 40 MFLP gels obtained, one dominant polymorphism was identified as a candidate marker linked to the *pauper* gene in the MFLP fingerprint generated by SSR-anchor primer MF129 in combination with AFLP primer *Mse*I-AGT (Fig. 1). In this particular MFLP fingerprint, all the 12 bitter plants exhibited a common band (112 bp in length), but this band was absent on all of the 12 sweet plants (Fig. 1).

Sequencing of the MFLP polymorphic band revealed that the candidate MFLP marker band was a 112 bp fragment. The sequence was extended for 324 bp beyond the SSR-end of the original *Mse*I-SSR fragment with the help of the *Taq*I-adaptor primer. A pair of sequence-specific primers, PauperM1F (5′-AAGAAAAGGCCCAATG-3′), and
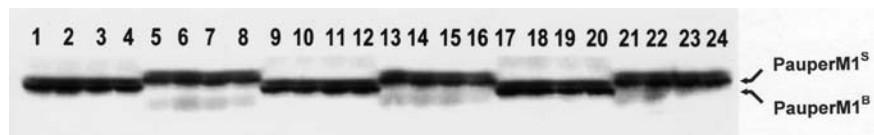
Fig. 2 Testing of a sequence-specific PCR-based molecular marker "PauperM1" with primer pair PauperM1F (5′-AAGAA AAGGCCCAATG-3′) and PauperM1R (5′-TTTTAAAGTCA-TACCATTGAG-3′) using un-digested genomic DNA as template. The 24 lanes indicated the same 24 plants which are illustrated in Fig. 1. Twelve tested plants were bitter, including the parent P27174 (Lane 1), RIL162 (Lane 2), RIL167 (Lane 3), RIL178 (Lane 4), RIL25 (Lane 9), RIL137 (Lane 10), RIL152 (Lane 11), RIL165 (Lane 12), RIL13 (Lane 17), RIL76 (Lane 18), RIL97 (Lane 19), RIL136 (Lane 20). The other 12 tested plants were sweet, including RIL7 (Lane 5), RIL68 (Lane 6), RIL72 (Lane 7), RIL89 (Lane 8), RIL3 (Lane 13), RIL61 (Lane 14), RIL87 (Lane 15), RIL100 (Lane 16), the sweet parent Kiev Mutant (Lane 21), RIL102 (Lane 22), RIL153 (Lane 23), and RIL195 (Lane 24). Marker "PauperM1" is co-dominant; the bitter-allele band "Pau-perM1[B]" is smaller (207 bp) and migrates faster in the gel than the sweet-allele band "PauperM1[S]" (209 bp)

PauperM1R (5′-TTTTAAAGTCATACCATTGAG-3′), was designed based on the obtained DNA sequence. The candidate MFLP marker was successfully converted into a sequence-specific PCR-based marker (Fig. 2). This sequence-specific PCR marker was designated as "PauperM1". PauperM1 is co-dominant; the bitter-allele marker band "PauperM1[B]" is 207 bp in length, which migrates faster in the gel than the sweet-allele marker band "PauperM1[S]", which is 209 bp (Fig. 2).

Confirmation of linkage

The 190 F_8 RILs from the cross Kiev Mutant × P27174 were tested with marker "PauperM1". A total of 94 RILs showed a homozygous marker band "PauperM1[B]" band, and the other 96 RILs showed a homozygous "PauperM1[S]" band (Fig. 2). Analysis by software MapManager (Manly et al. 2001) found that the marker "PauperM1" was linked to the low-alkaloid gene *pauper* at the genetic distance of 1.4 centiMorgans (cM). The LOD for this linkage was 47.2.

Marker validation

When marker PauperM1 was tested on the eight reference sweet varieties of *L. albus*, all the five varieties containing the *pauper* gene showed the homozygous marker band "PauperM1[S]". The three varieties containing the genes *exiguus* or *nutricius* showed the homozygous marker band "PauperM1[B]" (Table 2). Among the 13 bitter accessions, 2 lines, P25769 and P25787 from Greece, exhibited the homozygous band "PauperM1[S]". The other 11 bitter accessions showed the homozygous marker allele band "PauperM1[B]" (Table 2).

Among the breeding materials tested from the Australian breeding program at NSWDPI, all the 154 sweet lines possessing the low-alkaloid gene *pauper* showed the marker band "PauperM1[S]". All the 24 bitter genotypes showed the homozygous "Pau-perM1[B]" allele band. Of the seven "non-*pauper*" sweet genotypes, six showed the "PauperM1[B]" band, and one cultivar, Dietta, showed the marker band "PauperM1[S]". The results of a subset of the genotypes tested are given in Table 3.

Discussion

The successful development of marker PauperM1 in this study provides a powerful tool for lupin breeders to select plants possessing the gene *pauper* conferring low-alkaloid in *L. albus*. Testing on domesticated reference varieties found that marker PauperM1 is capable of distinguishing the targeted low-alkaloid gene *pauper* from the other two sweet genes *exiguus* and *nutricius*, which enables breeders to select progeny with the *pauper* genotype. Marker PauperM1 is closely linked to the gene *pauper* at 1.4 cM, which gives high accuracy in selection of the *pauper* genotype when it is used for MAS in lupin breeding. Marker PauperM1 possesses the characters of being PCR-based, simple, reliable, cost-efficient, and amenable to large numbers of samples, which are the key requirements for large scale marker implementation of MAS in plant breeding (Gupta et al. 1999; Eagles et al. 2001). Where fluorescence electrophoresis facility is available, labeling primers with fluorescence dye instead of radioisotope is equally successful for MFLP derived implementable markers (Yang et al. 2004), which makes marker implementation

Table 3 Validation of sequence-specific PCR marker "PauperM1" on local and foreign varieties and breeding lines from the Australian *L. albus* breeding program

Lines	Breeding status	Alkaloid genotype	"PauperM1" marker score[a]
Andromeda	Australian variety	Sweet, *pauper*	PauperM1[S]
Luxor	Australian variety	Sweet, *pauper*	PauperM1[S]
Magna	Australian variety	Sweet, *pauper*	PauperM1[S]
Minibean	Australian variety	Sweet, *pauper*	PauperM1[S]
Rosetta	Australian variety	Sweet, *pauper*	PauperM1[S]
Ultra	Australian variety[b]	Sweet, *pauper*	PauperM1[S]
WALAB2008	Australian variety	Sweet, *pauper*	PauperM1[S]
Hamburg	Foreign variety	Sweet, *pauper*	PauperM1[S]
Ida	Foreign variety	Sweet, *pauper*	PauperM1[S]
Madeira	Foreign variety	Bitter	PauperM1[B]
Minori	Foreign variety	Sweet, *pauper*	PauperM1[S]
Typtop	Foreign variety	Sweet, *pauper*	PauperM1[S]
Vladimir	Foreign variety	Sweet, *pauper*	PauperM1[S]
98B001-5-5	Breeding line	Sweet, *pauper*	PauperM1[S]
CH306-S	Breeding line	Bitter	PauperM1[B]
Esta-1-15	Breeding line	Sweet, *pauper*	PauperM1[S]
FP21	Breeding line	Sweet, *pauper*	PauperM1[S]
Lublanc-1	Breeding line	Sweet, *pauper*	PauperM1[S]
Lutop-1	Breeding line	Sweet, *pauper*	PauperM1[S]
Multolupa-2	Breeding line	Sweet, *pauper*	PauperM1[S]
W77	Breeding line	Sweet, *pauper*	PauperM1[S]
WK302	Breeding line	Sweet, *pauper*	PauperM1[S]
WK326	Breeding line	Sweet, *pauper*	PauperM1[S]
WTD180-1	Breeding line	Sweet, *pauper*	PauperM1[S]
LAB50001216	Breeding line	Sweet, *pauper*	PauperM1[S]
LAB50001231	Breeding line	Sweet, *pauper*	PauperM1[S]
LAB50001348	Breeding line	Sweet, *pauper*	PauperM1[S]
LAB50001362	Breeding line	Sweet, *pauper*	PauperM1[S]
LAB50001429	Breeding line	Sweet, *pauper*	PauperM1[S]
LAB50001443	Breeding line	Sweet, *pauper*	PauperM1[S]
LAB50001611	Breeding line	Sweet, *pauper*	PauperM1[S]
LAB50001687	Breeding line	Sweet, *pauper*	PauperM1[S]
Tuman	Foreign variety	Sweet, non-*pauper*	PauperM1[B]
Dietta	Foreign variety	Sweet, non-*pauper*	PauperM1[S]
Libed	Foreign variety	Sweet, non-*pauper*	PauperM1[B]
Veresnevy	Foreign variety	Sweet, non-*pauper*	PauperM1[B]
L80/91	Breeding line	Sweet, non-*pauper*	PauperM1[B]

[a] PauperM1[B] = bitter-allele marker band; PauperM1[S] = sweet-allele marker band

[b] A current Australian commercial variety that was originally bred elsewhere

more user-friendly. At present, marker PauperM1 is being implemented using radioisotope labeling at Department of Agriculture and Food Western Australia (DAFWA) in Perth; at the same time, this marker is also being implemented using fluorescence labeling at NSWDPI in Wagga Wagga of the Australian albus lupin breeding program.

The key factor determining the usefulness of a molecular marker for MAS in a plant breeding program is the extent to which the marker can be applied to multiple breeding populations (Holland 2004; Snape 2004; Yang et al. 2008). A molecular marker can only be used for MAS if the marker band is polymorphic between the two parents in a cross,

and the parent showing the target marker band does possess the target gene of interest, so that the desirable allele can be distinguished from the undesirable ones (Eagles et al. 2001; Sharp et al. 2001; Clements et al. 2005). Fortunately, marker PauperM1 developed in this study showed the banding pattern which is consistent with all the 154 cultivars and breeding lines containing the low-alkaloid gene *pauper*. The banding pattern is validated as correctly correlated with genotype on 35 out of the 37 bitter accessions, and on 10 out of 11 "non-*pauper*" sweet cultivars tested. The results indicate that marker PauperM1 is applicable to a wide range of crosses for MAS in lupin breeding programs for *L. albus*. Since marker PauperM1 is co-dominant, MAS by PauperM1 has the advantage of being capable of identifying homozygous individuals from heterozygous plants in F_2, which is impossible to achieve by conventional phenotyping methods, such as Dragendorff test (Harrison and Williams 1982) or the UV test (von Baer and Perez 1991).

Among the 37 bitter accessions of *L. albus* tested, 2 of them showed the "PauperM1[S]" allele marker band. Of the 11 "non-*pauper*" sweet cultivars tested, 1 cultivar, Dietta, showed the marker band "PauperM1[S]". The occurrence of such "false positives" is due to genetic recombination on a chromosome in the region between the marker and the target gene during evolution or during plant breeding process (Yang et al. 2008). When lupin lines with such "false positive" marker band are detected, breeders would be advised to avoid introducing such germplasm into the breeding program so that the marker's broad utility is preserved. If the germplasm was essential then special attention would be required for such crosses, or new markers should be sought closer to the target gene with a lower chance of recombination having occurred.

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