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SCAR markers linked to the common bean rust resistance gene *Ur-13*

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Abstract Rust in common bean (*Phaseolus vulgaris* L.) is caused by *Uromyces appendiculatus* Pers.:Pers. (Unger) which exhibits a high level of pathogenic diversity. Resistance to this disease is conditioned by a considerable number of genes. Pyramiding resistance genes is desirable and could be simplified by the use of molecular markers closely linked to the genes. The resistance gene *Ur-13*, present in the South African large seeded cultivar Kranskop, has been used extensively in the local breeding program. The purpose of this study was the development of a molecular marker linked to *Ur-13*. An F_2 population derived from a cross between Kranskop and a susceptible (South African) cultivar Bonus was used in combination with bulked segregant analysis utilizing the amplified fragment length polymorphism (AFLP) technique. Seven AFLP fragments linked significantly to the rust resistance and five were successfully converted to sequence characterized amplified region (SCAR) markers. The co-dominant SCAR markers derived from a 405 bp EAACMACC fragment, KB126, was located 1.6 cM from the gene. Two additional SCAR markers and one cleaved amplified polymorphic sequence marker were located further from the gene. The gene was mapped to linkage group B8 on the BAT 93/Jalo EEP 558 core map (chromosome 3).

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Introduction

In recent years, increasing use has been made of molecular markers for the tracking of disease resistance genes in numerous agricultural crops. For the common bean (*Phaseolus vulgaris* L.), genetic markers are available for a wide range of disease resistance genes. These include random amplified polymorphic DNA (RAPD) or sequence characterized amplified region (SCAR) markers for seven rust [*Uromyces appendiculatus* Pers.:Pers (Unger)] resistance (RR) genes (Kelly et al. 2003; Miklas et al. 2002; Park et al. 2003), as well as various types of molecular markers for genes conveying resistance to anthracnose (*Colletotrichum lindemuthianum*; reviewed by Kelly and Vallejo 2004), angular leaf spot (*Phaeoisariopsis griseola*), *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) (reviewed in Kelly et al. 2003).

The amplified fragment length polymorphism (AFLP) technique has not yet been widely used for the tracing of disease resistance in bean. However, Naidoo et al. (2003) have used this technique to identify markers linked to common bacterial blight (caused by *Xanthomonas axonopodis* pv. *phaseoli*) (CBB) resistance, and Kolkman and Kelly (2003) to identify quantitative trait loci (QTL) conferring resistance to white mold (caused by *Sclerotinia sclerotiorum*). QTL markers have also been developed for ashy stem blight (caused by *Macrophomina phaseolina*), *Bean golden mosaic virus* (BGMV), CBB and web blight (caused by *Rhizoctonia solani*) (Kelly and Miklas 1999; Kelly et al. 2003).

In the case of applying markers in breeding for resistance to bean rust, the RAPD marker $OA14_{1100}$ was used to detect the *Ur-4* gene (Miklas et al. 1993) in the presence of the *Ur-11* gene (epistatic to *Ur-4*) in Bel-MiDak-RR-lines (Kelly et al. 1993; Stavely et al. 1994). RAPD markers were also used to confirm the presence of *Ur-3* and the *I* gene for BCMV resistance in Bel-DakMi-RMR-14 (Stavely et al. 1998). Corrêa et al.

(2000) validated utility of flanking markers for transferring RR from the cultivar Ouro Negro to advanced dry bean breeding lines. The breeding programme in Vicosa, Brazil, routinely uses $AE19_{890}$ RAPD marker (Johnson et al. 1995) to assist transfer of *Ur-11* into Carioca bean cultivars (Souza et al. 2003). Interestingly, $AE19_{890}$ is linked in repulsion and selected against to retain *Ur-11*. As with most useful RAPD markers it was recently converted to a SCAR marker to facilitate utility for marker-assisted selection (Queiroz et al. 2004).

In view of the high level of pathogenic diversity within *U. appendiculatus* (Stavely et al. 1989; Pastor-Corrales 2001), the pyramiding of RR genes in dry bean cultivars is becoming increasingly desirable. The majority of the South African large seeded red speckled sugar (cranberry) cultivars share a narrow genetic base, having been bred from the high yielding and widely adapted cultivar Kranskop. The RR of some of these cultivars is presently being improved by means of backcrossing. However, it is desirable that the useful but vulnerable RR gene common to this group (*Ur-13*), which is hypostatic to the genes being introgressed, be retained. *Ur-13* is of international importance as it gives protection to many races of *U. appendiculatus*. *Ur-13* is also present in the rust differential cultivar Redlands Pioneer (Liebenberg and Pretorius 2004) which was reported resistant to moderately resistant in many parts of Latin America, the USA, Australia and Africa (Schwartz 1980) resistant to all 39 races identified in Brazil (Alzate-Marin et al. 2004), and also to many internationally reported races (summarized in Liebenberg 2003). The purpose of this study was the identification of a molecular marker(s) linked to *Ur-13* that would enable the tracking and preservation of this resistance in future crosses.

Materials and methods

Population development

A segregating F_2 population was developed from a cross between the South African cultivars Bonus (susceptible parent: female) and Kranskop (resistant parent: male) (Liebenberg and Pretorius 2004). Young leaf samples were collected from 107 F_2 plants, freeze dried and stored separately for each plant at −20°C. The F_2 plants were allowed to self-pollinate to produce F_3 seed progenies. F_3 progenies (17 to 40, with an average of 33 plants/progeny) were screened for rust resistance to determine genotype *Ur-13*//*Ur-13* (R), *Ur-13*//- (R), or -//- (S) of F_2 plants.

Rust inoculation and scoring

Leaf stage, inoculum concentration, inoculation method, treatment of plants and disease rating were as previously described (Stavely 1983; Liebenberg and Pretorius 2004). The South African race RSA-Ua7 (Liebenberg 2003), to which Kranskop is resistant and Bonus susceptible, was used to screen breeding material. RSA-Ua7 overcomes the Andean genes *Ur-4* and *Ur-6*, but not the Middle-American genes *Ur-3*, *Ur-5* or *Ur-11* (Liebenberg 2003). For the purpose of genetic analysis, a rating of "3" (pustules averaging approximately 0.3 mm in diameter, with none larger than 0.5 mm) was considered a resistant reaction, and a rating of "6" (pustules generally 0.6 mm and larger in diameter), a susceptible reaction.

AFLP analysis

DNA was isolated from lyophilised leaf material of F_2 plants by a modified CTAB extraction procedure (Dellaporta et al. 1983) and utilised in bulked segregant analysis using the AFLP technique. Two bulks were constructed using equal amounts of DNA from each of ten homozygous susceptible and ten homozygous resistant plants, confirmed by F_3 progeny tests. DNA from the parents and contrasting bulks were screened for polymorphisms with a range of primer combinations. A slightly modified version of the protocol developed by Vos et al. (1995) was followed, employing *Eco* RI and *Mse* I as rare- and frequent-cutter enzymes, respectively. The procedure included a pre-amplification step with primers having a single selective nucleotide, followed by a second amplification with primers having three selective nucleotides as described by Vos et al. (1995). The protocol was modified in using 30 ng of all AFLP primers without radioactive labelling.

PCR products (3 µl) were separated on a 5% (m/v) denaturing polyacrylamide (19 acrylamide:1 N,N′-methylene-bis-acrylamide ratio) gel containing 7 M urea and using 1×TBE buffer (89 mM Tris-borate, 2.5 mM EDTA, pH 8.3) as running buffer. Electrophoresis was carried out at 80 W constant power for approximately 2 h using a standard DNA sequencing unit (C.B.S. Scientific Company, California, USA). The separated amplified DNA fragments were visualized with a silver staining kit from Promega according to the manufacturers instructions. The gel was air dried and photographed by exposing photographic paper (Kodak Polymax II RC) directly under the gel to approximately 20 sec of dim light, to produce a negative image of the same size as the gel.

Polymorphisms observed between the parents and bulks were verified by amplification of the DNA of the individual plants comprising the two bulks. The frequency of informative bands was also determined in an additional 40 plants from the F_2 population.

SCAR development

To improve readability, SCARs have been assigned shortened names (for example $SEAACMACC_{405}$ is

Fig. 1 Sequences of AFLP fragments used in the development of SCAR markers linked to the *Ur-13* gene conditioning resistance to *Uromyces appendiculatus* in the dry bean cultivar Kranskop. AFLP primer sequences are indicated in bold and the positions of SCAR primers are underlined

SEAACMACC$_{405}$ (KB126)

GACTGCGTACCAATTCAACCTCGGCCACTACCATAGGTTTCCAACGAATGGACACTGAGCGTCTGCGTTTTCTCTTTCTTG
AACAGCAGCCAGCATCAGGCATGATCTTCTGAACAGGAGATACAGGTTCCTCCAGCCAAATAAGTTTCTTCTCTGAGGGTC
CATCCATCAAAGGATCTCTATCGTATTCCTCTTGAAAGCAAATGATGCTACCACTTTTTTTATTGCTATCCAACAAGTTTGAC
ATGTCGCCATTTTCCATAGGGATATCGGAAACAATAATGTCAGAACCAAATACGTTAGTGTGTGCAGCATCACAGTGAATGT
TGTTGTCAGTAGGTTCAACTGAGTTCAGTTCTTGTGTTTCTTTCNAGGAATCCTCCGGAA**GGTTTACTCAGGACTCATC**

SEACAMCTT$_{320}$ (KB85)

GATGAGTCCTGAGTAACTTCATACTTGTTCTGAGTCATTTACCTTGCATTCTGTCTTTTTCCTTCTTTGTTCATTTTGATAATT
AGTGCTCTCCAAGTATATCTAATGAAAAACAATGAAATTGAAGTCAGCATTGTTTCTCTTCTGTTTGCTGATAATAATTGTTG
AATAAGTTCTAACTTATGGAACACGACAATAAAAGCTTTATATCTTTAGTGGAAATAAAGAGCATAACATGTTATCAAGAAAT
TTCCTAAGCAAGATTTGTGATTGATCCAGATGGAAGATTAGAGTAGTTTGCAC**TGTGAATTGGTACGCAGTC**

SEAAGMCGT$_{480}$ (KB4*Hha* I)

GATGAGTCCTGAGTAACGTGGAGGCCCTGTTCATCCAGCGGCCTACTAATTACCTGTATAGGCCCATTGGAAGGCCCATA
GTTGGAGCCCGAGAATACCCATAAAAAATCACGAAAATAGAAAAAAGTAAAAAAAGAGGTAGTGCAAGGCTGGGTTTTGTA
GCAGAAAGAAAGGGTTTTTCATTTCGCAAACACCGGTCGCACTCTTGTCTCCAGGGATTCCATTATCTCTGCTCTCACTTCA
AAGCTTTTCTCTGCTCTATCAGGTACTATTCATTTTCGCCTTTCAATATCTGTTCCTATTATTTTTCTCCTTTTTTGTTTCTGTT
CTCCGATGTTTGTACCTAATCAATTTCAATTGATTTTTTCGTTTTTCGCTCTTCCACGTCCAAAATATGTGCTATTTATTGTGT
TTTATCCATCCATTTCCAGAATCCGTTTCGGTGCGTGCTT**CTTGAATTGGTACGCAGTC**

SEAAGMACT$_{130}$ (KB7)

GATGAGTCCTGAGTAAACTACCATTCATGCATGTAATGTCCTATTAGAAAAGGAGTTTGGAAATAGCTTAGATAAGGTTCTA
AGGATTTTTTTTTCCCAACTCTTGTTTG**CTTGAATTGGTACGCAGTC**

designated as KB126). Conventional names of potentially useful markers are given in Fig. 1.

After silver staining, putative marker fragments were excised from the dried polyacrylamide gel (Cho et al. 1996) and reamplified directly from the gel segment without any purification, using the same conditions as for the original AFLP reaction.

The PCR products were purified using the Wizard® PCR preps DNA purification system (Promega Corporation) and cloned into the pGEM® T-Easy vector, following the manufacturers recommendations. One to five clones of each fragment were sequenced by the Central DNA Sequencing Facility, University of Stellenbosch, South Africa. All sequences contained the *Eco* RI adapter at one end and the *Mse* I adapter at the other end. New primers internal to the 5' and 3' ends of the fragment were designed using the NetPrimer program of PREMIER Biosoft International (http://www.PremierBiosoft.com). Primers were synthesised by GibcoBRL or Qiagen OPERON. Amplification conditions for the designed primers were determined empirically by varying annealing temperature and $MgCl_2$ concentration. Reactions were carried out in 25 µl containing 25–50 ng genomic DNA, ×mM $MgCl_2$, 0.2 µM each of forward and reverse primers, 200 µM each of dATP, dCTP, dGTP and dTTP, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1% (v/v) Triton X-100 and 1 U *Taq* polymerase (Promega Corporation). Amplification was done in a Hybaid Thermal Cycler (Hybaid Limited, United Kingdom) with a denaturation step at 94°C for 5 min, followed by 35 cycles, each cycle consisting of 94°C for 1 min, determined annealing temperature for 1 min and 72°C for 1 min. A final elongation step of 5 min at 72°C was included in the programme. All SCAR reactions standardised at 2 mM $MgCl_2$, with KB126, KB1, KB7 and KB85 annealing optimally at 45°C and KB2 and KB4 at 60°C. Segregation and linkage of SCARs were verified by amplification of DNA from the total number (107) of individuals in the population.

Statistical data analysis

Chi square was used to test the goodness of fit for obtained ratios. P-values were obtained using BIOMstat Version 3.30 (© 1996–2002 Applied Biostatistics, Inc.). Data from the 107 F_2 plants were analysed with a general linear model (analysis of variance using categorical data) of the STATGRAPHICS Plus computer program (Manugistics, Rockville, Maryland, USA, 1998) using genetic marker data as the independent and rust disease ratings as the dependent variable. The association between the DNA marker and the trait was considered significant where the probability was ≤ 0.05. The coefficient of determination (R^2) was used as a measure of the magnitude of association. Interval mapping with MAPMAKER-EXP (Lincoln et al. 1992) was used to link the markers on a genomic map of the Bonus/Kranskop cross. Linkage data were used to assign markers to linkage groups if the LOD was ≥3.0 and the distance was ≤ 80 cM with the Kosambi mapping function.

KB126 was amplified in the BelNeb-RR-1/A55 population (developed by Ariyarathne et al. 1999) (KB4 *Hha* I and KB85 were not polymorphic). KB4 *Hha* I and KB85 were mapped on the BAT 93×Jalo EEP 558 mapping population (developed by Freyre et al. 1998). Due to technical problems arising from differences between laboratories, marker KB126 could not yet be mapped in this population, although it was polymorphic in the parents (Liebenberg 2003).

Results

Identification of markers linked to the rust resistance gene

DNA from parents and contrasting bulks were screened for informative polymorphisms with a total of 144 primer combinations. AFLP bands present in both the resistant parent and resistant bulk, and absent in the susceptible parent and susceptible bulk, or vice versa, were regarded as candidate markers. Sixteen putative AFLP markers were identified with bulked segregant analysis and these primers were used to analyse the twenty individuals comprising the bulks to determine whether there was significant linkage to the resistance trait. Four of the markers ($EAACMACC_{405}$, $EACTMTTG_{160}$, $EAGCMACC_{400}$, and $EAGCMTAC_{360}$) revealed a 100% linkage in the individual plants comprising the contrasting bulks. Verification of the segregation pattern of the markers relative to the trait in an additional 40 individual plants from the F_2 population, confirmed tight linkages for seven of the AFLP fragments.

SCAR development

Five AFLP fragments were successfully cloned and sequenced, and SCAR primers designed. Sequences for four of these are shown in Fig. 1. Several sets of primers were tested for AFLP fragment $EAACMACC_{405}$. SCAR KB126 was 1.6 cM from the gene. The marker produced two polymorphic fragments in the parents (Fig. 2a), indicating either a deletion or insertion mutation between the two alleles. The fragment amplified in the resistant parent was the same length (405 bp) as the original AFLP fragment with a slightly larger fragment (430 bp) amplified in the susceptible parent. The original AFLP and co-dominant SCAR mapped to the same location on the linkage map. The fragments segregated close to a 1:2:1 ratio ($p = 0.47$) (Table 1) thus acting as a co-dominant marker. SCAR marker KB85, developed from the AFLP fragment $EACAMCTT_{320}$ produced two polymorphic bands between the two parents (Fig. 2b) and also segregated close to a 1:2:1 ratio ($p = 0.29$) (Table 1), acting as a co-dominant marker. The amplification conditions for KB126 and KB85 were identical and these two primer sets could be multiplexed. The non-polymorphic PCR products of three markers (KB1, KB2 and KB4) were screened with several restriction enzymes. A polymorphism was detected with *Taq* I in KB1, resulting in several fragments, with two fragments being polymorphic (210 bp and 190 bp) between the two parents, thus acting as a co-dominant marker. SCAR marker KB2 digested with *Hae* III was also polymorphic in the mapping population, but both KB1 *Taq* I and KB2 *Hae* III displayed weak linkage to the resistance trait (37.6 cM and

Table 1 Development of SCAR markers from isolated AFLP fragments linked to the rust resistance gene *Ur-13* in the dry bean cultivar Kranskop

SCAR (abbreviated name)	Primers (*Eco*RI-*Mse* I)	Size (bp)	R^2 (%) ($p \leq 0.05$) (107 plants)	Expected ratio	Chi^2 (p)	SCAR primers	Phase[1]	PCR product (bp)
KB1 *Taq* 1	AAC-ACC	405	16.0	1:2:1	0.09(0.96)	F:5′-CTTAAGTTGTCTGGTCAA R:5′-AATTTGGTTGGTTACTTC	Co	190, 210
KB4 *Hha* I	AAG-CGT	480	51.4	1:2:1	2.57(0.28)	F:5′-TAACGTGGAGGCCCTGTTCATC R:5′-CACGCACCGAAACGGATTCTGG	Co	436, 250 + 186
KB7	AAG-ACT	130	66.6	3:1	0.15(0.70)	F:5′-GCATGTAATGTCCTATTAGAA R:5′-CAAGAGTTGGGAAAAAA	C	78
KB85	ACA-CTT	320	70.8	1:2:1	2.45(0.29)	F:5′-TTAACTTCATACTTGTTCTG R:5′-GAATTCACAGTGCAAACT	Co	310, 288
KB126	AAC-ACC	405	91.1	1:2:1	1.53(0.47)	F:5′-GAATTCAACCTCGGCCACTACC R:5′-TTAAACCTTCCGGAGGATTC	Co	430, 405

[1] C = Coupling phase linkage; Co = Co-dominant

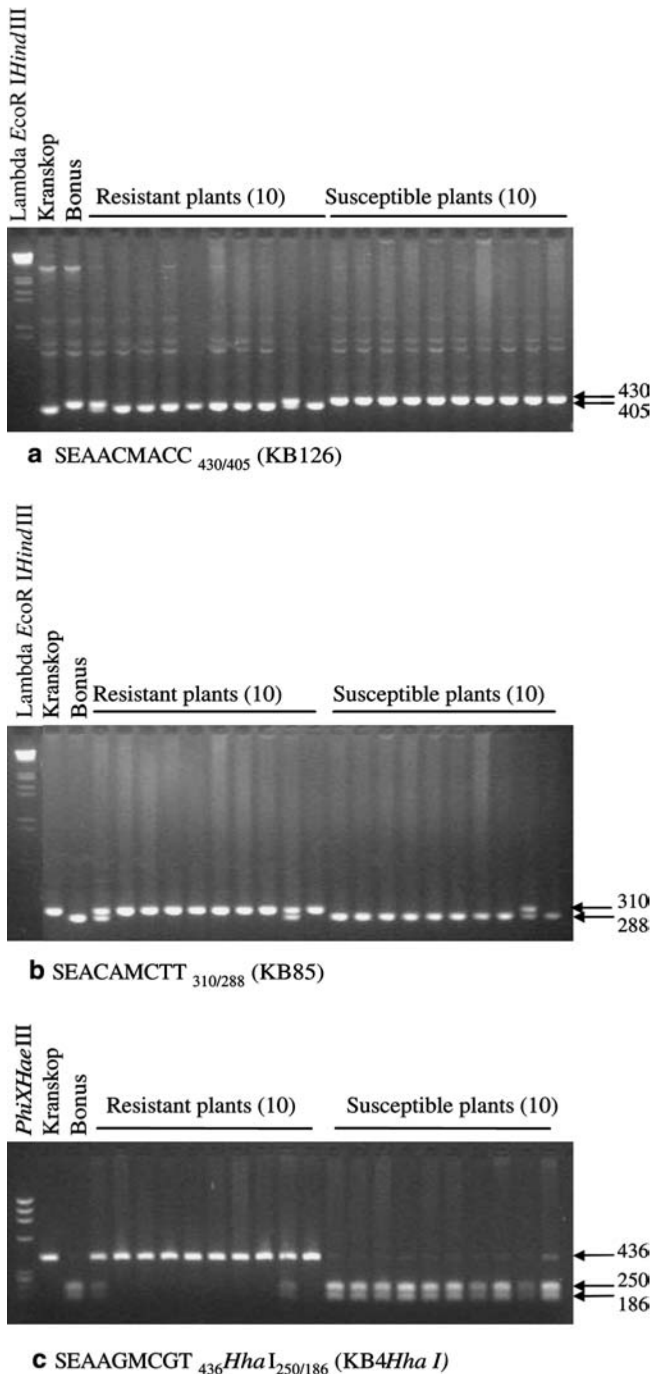

Fig. 2 Segregation patterns of SCAR markers KB126 (a), KB85 (b) and KB *Hha* I (c) linked to the rust resistance gene *Ur-13*. Amplified fragments were electrophoresed in 3% Nusieve GTG agarose

40.5 cM, respectively). Marker KB4 was polymorphic after restriction digestion with *Hha* I (16.1 cM from *Ur-13*) and also resulted in a co-dominant marker (Fig. 2c). KB4 *Hha* I and KB126 flanked *Ur-13*. The amplified fragment (436 bp) of the resistant parent (Kranskop) did not have an *Hha* I site, whereas Bonus (susceptible parent) produced two fragments (250 bp and 186 bp). This indicated a point mutation between the two alleles. Relatively tight linkage (12.8 cM) was obtained with KB7, which acted as a dominant marker linked in coupling phase. The marker segregated in a 3:1 ratio (Chi square = 0.15, p = 0.70). Attempts to convert several other AFLP fragments (not listed) tightly linked with *Ur-13* to SCAR markers resulted in non-polymorphic bands, which could not be converted to cleaved amplified polymorphic sequence (CAPS) markers with the limited restriction enzyme set screened.

Mapping of the markers

Altogether 31 AFLP and SCAR markers were assayed across the F_2 population consisting of 107 individuals. The majority of the markers mapped on the same linkage group (Fig. 3) as would be expected when using the bulked segregant analysis approach. SCAR marker KB126 mapped to the same location as the original AFLP marker ($EAACMACC_{405}$), 1.6cM from the resistance locus. KB85 mapped to the same location as the original AFLP 9.3 cM from the resistance gene on the same side as KB126 relative to the gene. KB7 was located a further 3.6 cM away. Marker KB4 *Hha* I flanked the resistance gene, mapping 14.7 cM away, but was further from the gene than the original AFLP marker, indicating that it amplified a different fragment from the genome. SCARS KB1 *Taq* I and KB2 *Hae* III mapped to the same region as KB4 *Hha* I, but were relatively distant from the resistance gene.

KB126, KB4 *Hha*I and KB85 were co-located on linkage group (LG) B8 of the BAT93/Jalo EEP 558 core map (Freyre et al. 1998) (Fig. 3); KB126 in the BelNeb-RR-1/A 55 mapping population (Ariyarathne et al. 1999), and the latter two directly in the BAT 93/Jalo EEP558 core mapping population. The distance between KB4 *Hha*I and KB85 was similar in the Bonus/Kranskop (23 cM) and BAT 93/Jalo EEP 558 (20.8 cM) maps. Although the location of KB126 on the core map via the H11.400 RAPD marker is inferred, this location tallies with its position on the Bonus/Kranskop map. Collectively the mapping results integrate *Ur-13* in the vicinity of the anchor RFLP marker Bng73 (Vallejos et al. 1992), located toward the end of LG B8 (syn. chromosome 3) (Fig. 3). Linkage groups were recently assigned chromosome designations by Pedrosa et al. (2003), and Bng73 was located on the long arm of chromosome 3 in the F-linkage group (as opposed to the F'-linkage group located on the short arm of chromosome 3).

Discussion

In this study, a bulked segregant analysis approach (Michelmore et al. 1991) was followed in which the two extreme bulk samples provided a crude simulation of

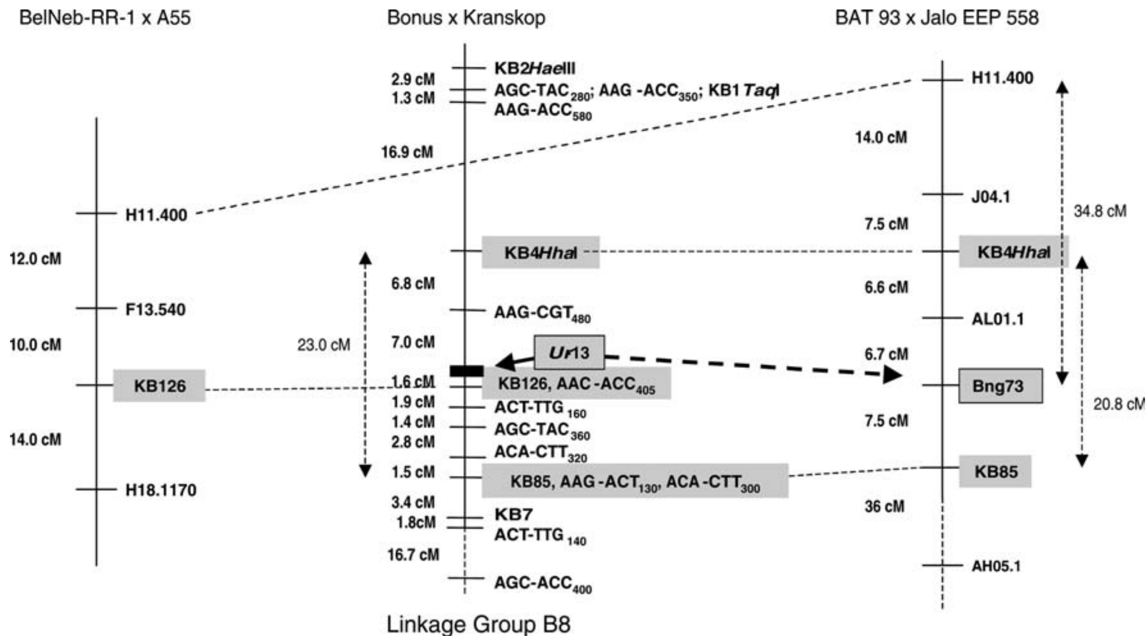

Fig. 3 Partial linkage map for *Phaseolus vulgaris*, integrating newly developed AFLP and SCAR markers for *Ur-13* and existing DNA markers and indicating the relative location of the rust resistance gene *Ur-13* on linkage group B8 of the BAT 93/Jalo-EEP 558 core map (not to scale). For marker names, the first three letters are prefaced by "E" and the second three, by "M"

near isogenic lines (NILs). A process of elimination of unlinked markers minimized the number of samples to be tested. This approach was highly efficient for identifying associated markers, especially when dealing with a single dominant gene (Liebenberg and Pretorius 2004). Although AFLP is a powerful technique for the identification of associated markers, the relatively long and costly procedure makes it economically unacceptable for routine application in plant breeding programmes. Conversion of the relevant AFLP fragments into sequence-specific PCR markers is therefore necessary. Three potentially useful SCAR markers were developed for *Ur-13*. KB126 and KB85 are co-dominant insertion-deletion markers, with the CAPS marker KB4 *Hha* I also able to differentiate between homo-and heterozygotes. Of these markers, the KB126 SCAR mapped closest to the resistance gene (1.6 cM). All three markers, but in particular KB126 and KB4 *Hha* I, which flank *Ur-13*, will find application in identifying those improved breeding lines (containing epistatic RR genes) which retain *Ur-13*. Although KB85 is of less value, being further from the gene, it is easy to apply (combined with KB126 in a multiplex reaction), and enables the selection of plants with or without the additional DNA segment, depending on the advisability of including more or less of the Kranskop genome. The co-dominant nature of all three markers confers the added benefit of distinguishing plants homozygous and heterozygous for *Ur-13* (Fig. 2).

All three markers can be used when more detailed information is needed about the genetics of a specific cross, and all three have been useful in locating the gene on the core mapping population. The integration of *Ur-13* on LG B8 of the core map confirms earlier conclusions based on race inoculations and field observations (Liebenberg 2003; Liebenberg and Pretorius 2004) that *Ur-13* is independent from other known rust resistance genes.

An added application for these markers is their usefulness in tracing the source of the RR present in the Kranskop group (Liebenberg 2003), as well as the identification of other cultivars which may have the same resistance, simplifying the future choice of crossing material, and adding to our understanding of common bean germplasm. For instance, *Ur-13* appears to be present in Redlands Pioneer (Liebenberg and Pretorius 2004), which has been widely used as an international rust differential cultivar.

The clustering of disease resistance genes within linkage groups has been reported for several crops, for instance lettuce (*Lactuca sativa*) (Witsenboer et al. 1995), soybean (Ashfield et al. 1998) and sugar cane (S *accharum officinarum*) (Rossi et al. 2003). Clustering is very pronounced in *P. vulgaris* with major clusters located on linkage groups B1, B4, B7, and B11 for resistance to various diseases including rust, anthracnose, common bacterial blight (CBB), bean golden yellow mosaic virus, and angular leaf spot (Geffroy et al. 1999; Miklas et al. 2002; Kelly et al. 2003; Vilarinho et al. 2003). No other rust resistance genes have yet been reported on LG B8, the only other rust-related gene on this group being "*Crg*" (*C*omplements *r*esistance *g*ene), necessary for the expression of *Ur-3* (Kalavacharia et al. 2000). However, this gene is located at the other end of B8, near a cluster of four resistance gene analog (RGA) sequences and the Bng139 anchor marker (Vallejos 1994; Rivkin et al. 1999; Kalavacharia et al. 2000). Several cases are known where major genes and QTL

conditioning resistance to different diseases cluster together. On LG B4, several major rust, anthracnose, and halo blight (Fourie et al. 2004) resistance genes cluster with QTL conditioning resistance to ashy stem blight, BGMV and anthracnose (Kelly et al. 2003). B7 contains a cluster including the *Bct* gene conditioning resistance to *Beet curly top virus* (Larsen and Miklas 2004), an anthracnose resistance gene, and QTL conditioning resistance to CBB, white mold, BGMV and ashy stem blight (Kelly et al. 2003). For B8, QTL conditioning resistance to CBB (Jung et al. 1997; Miklas et al. 2000) and white mold (Park et al. 2001) have been reported in the vicinity of the Bng73 anchor marker (Kelly et al. 2003). The possible linkage of *Ur-13* with these traits needs to be investigated. Other disease resistance genes and QTL located on B8, such as the *Co-4* gene conditioning resistance to anthracnose associated with linkage group F' on the short arm of chromosome 3 (Melotto et al. 2004), are not close enough to be clustered with *Ur-13*.

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