CONVERSION OF THE RAPD MARKER FOR UR-4 TO A CO-DOMINANT SCAR MARKER $SA14_{1079/800}$

C.M.S. Mienie, R. Naidoo and M.M. Liebenberg

Agricultural Research Council-Grain Crops Institute, Private Bag X1251, Potchefstroom, 2520, South Africa

Rust is an important disease of dry bean, and efforts are being made to improve the rust resistance (RR) of South African cultivars. Due to epistasis, it is sometimes difficult to detect the presence of multiple genes in breeding lines. Marker assisted selection makes this possible and the $OPA14_{1100}$ marker has been used for the detection of the dominant RR gene *Ur-4* in the presence of *Ur-11* (Kelly *et al.* 1993; Stavely *et al.* 1994a). *Ur-4*, which appears to be of Andean origin, as it occurs in all Andean material tested (Miklas *et al.*, 1993) was tagged by Miklas *et al.* (1993) with the RAPD marker $OA14_{1100}$ and has since been mapped to linkage group B6 (Miklas *et al.*, 2002). No recombination was observed between the marker and the resistant allele in the mapping population. Although the marker was reported to be repeatable and easy to score (Miklas *et al.* 1993), it proved difficult to work with under local conditions. It was therefore decided to develop a SCAR marker from the RAPD fragment.

Materials and Methods

The fragment was isolated, cloned using a pGEM T-easy kit (Promega), transformed into bacterial cells (JM109) and sequenced by the central sequencing facility at Stellenbosch University, South Africa. The sequence of the 1127bp fragment is given in Figure 1. New primers (SA14-F: 5'-CTA TCT GCC ATT ATC AAC TCA AAC-3' and SA14-R: 5'-GTG CTG GGA AAC ATT ACC TAT T-3') were deducted from this sequence and the new reaction was optimized. PCR was conducted in a final volume of 20 µl containing approx. 100 ng genomic DNA, 30 ng each of forward and reverse primers, 2 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP and dTTP, 1 x reaction buffer (10 mM Tris-HCl (pH 9), 50 mM KCl, 0.1% Triton® X-100) and 1 unit *Taq* polymerase (Promega, Madison, WI). Amplification was performed in a Hybaid thermal cycler or Hybaid Omnigene for one cycle at 94°C for 5 minutes; followed by 35 cycles of 94°C for 1 minute, 55°C for 1 minute and 72°C for 1.5 minutes. A final elongation step of 72°C for 5 minutes was included. Amplification products could be separated on 2% agarose (Seakem LE) for 1 hour at 80V.

Results

The SCAR marker acted co-dominantly, amplifying two different bands for the two alleles present at this locus (Figure 2), with a fragment of 1079 bp linked to the resistant allele (in BelMiDak-RR-9 and -RMR-11) and 800 bp linked to the susceptible allele (in Teebus and BelNeb-RR-1). The results were repeatable and easy to score. Our results confirmed the finding of Miklas *et al.* (1993) that the RAPD marker was only useful in a Middle-American genetic background. All cultivars of Andean origin were positive for the 1079 bp allele (resistance allele), except for KW780 (Liebenberg *et al.*, 2004). The SCAR marker mapped to exactly the same position as the RAPD marker (Miklas, personal communication, April 2002) on linkage group B6.

5'-**TCTGTGCTGG**TAGTTTTCCTTTAGAAGCTAAGATCTGGAATTTGGCTATCTGCCATTATCAACTCAAACGCAT
CATAAATTTTTTGAGTCAATTGTGTGTTTTGCTAAGAATTTTGGTATTCAATGTTGCAGACTAGAAATCTTCTTTT
GCTGGTGTTTTCTTCTCATTAATGGTGAATTTTTTTTATTATTGTTATTGTTATTTAGATATTTAACAACAATTACT
TTACTTTGTGTAGGTACTTGATAATCTTCCACATGATCTTATCTATGCAGAGAATCAAATTTCCTCATGGATGGAA
GTCTGGGTTGAGAAGCAGCATGACCAGTAAGTCTAAGCTTTATGTGTGGATGCTAAATATACTGGTACATATTCC
AGTGCCTCATTGGGAGTAGCTGTTAGAAAATCTGACAAATATCTCATGATATTTTTTTATATATCTTAGAATATTT
TAGAATATTCTAGATGATATTTTTTATTTTATGATTTTTGCTTTCTTATTTTTTATGGGGTTGAGTTAGGCTTAAAG
TCCACTTTGTAATATGGTATCAGAGCCCATTTCGAGTCTATCCTAGCGAGTATTTGTGTTGGGCCTATCGTGCCAC
CCGCTATCGGATCACCCATAATATATAGTCTCACGCACGAGTTGGCAGTCTCGGCGTGAGGGGGGTGTGTTGGAG
ATCCCACATCGACTAGAGATTAGAGTCTTTCATTGTATATAAGTGGGTGCAAATCTCAACTCTATGAGCCGGTTTT
ATGGGGTTGAGTTAGGCTTAAAGTCCACTTTGTAATATATTTAATCAAGATCTTTGTAATGATAGGTCTAATCATA
TCATATGAATAGGAATATTATTTCGTATATTTATTTGTATTTGTTTCATTAACTCTATATAAAACGCACTGAAATAT
TGTGTACTCAGTTTCAATATCTCTTGCATCTATTTTTCTCTTTAAAAACATCATATTAGAGCTGTACCAATTAGTCTC
CTCTTCCATAGTGTCTCTTCTTTTCAATTTGACTATCATTTTTCGGCTATGTCCTAATTTGGTTTACACTGGAAAAAT
CCATGGCAAATGTTGTTCACCAAGATTGACTTTCCCAATAGGTAATGTTTCCCAGCACAGA-3'

Figure 1 Sequences of the 1127 bp fragment of the RAPD product of OPA-14. The RAPD primers are indicated in bold type and the location of the SCAR primers is underlined.

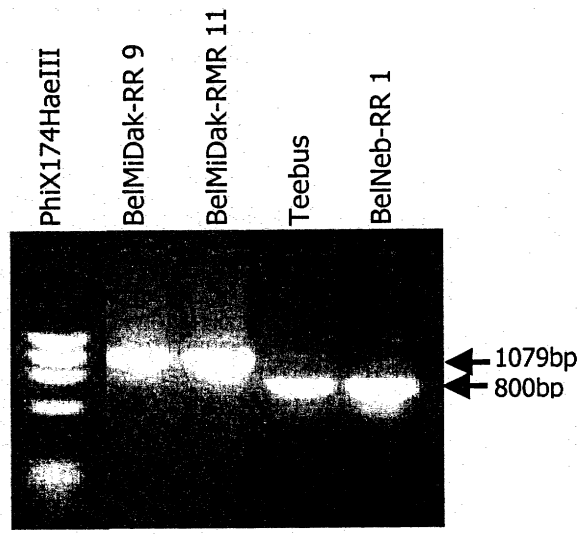

Figure 2 PCR amplification patterns of negative controls Teebus and BelNeb-RR-1 and positive controls BelMiDak-RR-9 and BelMiDak-RMR-11 with SCAR primers $SA14_{1079/800}$. PCR products were separated on a 2% agarose gel at 80 V for 2 hours and visualized with ethidium bromide.

References

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