Theor Appl Genet (1998) 96 : 1162–1169 © Springer-Verlag 1998

A. A. Myburg · M. Cawood
B. D. Wingfield · A-M. Botha

Development of RAPD and SCAR markers linked to the Russian wheat aphid resistance gene *Dn2* in wheat

Received: 1 July 1997 / Accepted: 20 October 1997

Abstract RAPD (random amplified polymorphic DNA) analysis was used to identify molecular markers linked to the *Dn2* gene conferring resistance to the Russian wheat aphid (*Diuraphis noxia* Mordvilko). A set of near-isogenic lines (NILs) was screened with 300 RAPD primers for polymorphisms linked to the *Dn2* gene. A total of 2700 RAPD loci were screened for linkage to the resistance locus. Four polymorphic RAPD fragments, two in coupling phase and two in repulsion phase, were identified as putative RAPD markers for the *Dn2* gene. Segregation analysis of these markers in an F_2 population segregating for the resistance gene revealed that all four markers were closely linked to the *Dn2* locus. Linkage distances ranged from 3.3 cM to 4.4 cM. Southern analysis of the RAPD products using the cloned RAPD markers as probes confirmed the homology of the RAPD amplification products. The coupling-phase marker $OPB10_{880c}$ and the repulsion-phase marker $OPN1_{400r}$ were converted to sequence characterized amplified region (SCAR) markers. SCAR analysis of the F_2 population and other resistant and susceptible South African wheat cultivars corroborated the observed linkage of the RAPD markers to the *Dn2* resistance locus. These markers will be useful for marker-assisted selection of the *Dn2* gene for resistance breeding and gene pyramiding.

Key words Wheat · Russian wheat aphid · *Dn2* resistance gene · RAPD · SCAR

Communicated by J. Beckmann

A. A. Myburg[1] · M. Cawood · A.-M. Botha (✉)[2]
Department of Botany and Genetics, Faculty of Science, University of the Free State, PO Box 339, Bloemfontein, 9300, South Africa

B. D. Wingfield[2]
Department of Microbiology and Biochemistry Faculty of Science, University of the Free State, PO Box 339, Bloemfontein, 9300 South Africa

Present addresses:
[1]Forest Biotechnology Group, Department of Forestry, Box 8008, North Carolina State University,
North Carolina, 27695-8008, USA
[2] Department of Botany and Genetics, Faculty of Biological Sciences and Agriculture, University of Pretoria, Pretoria, 0002, South Africa
Fax: +27 12 420 3947
E-mail: ambothao@scientia.up.ac.za

Introduction

Large-scale damage to small-grain crops caused by infestation with the Russian wheat aphid (*Diuraphis noxia* Mordvilko) has been reported since its introduction to South Africa (Hewitt et al. 1984), South America, Mexico (Gilchrist et al. 1984), the United States (Webster et al. 1987) and southwestern Canada (Morrison 1988). Crop damage in the USA alone amounted to $850 million for the period 1987–1992 (Brooks et al. 1994). The severe damage caused by *D. noxia* in wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) has necessitated the routine application of expensive and environmentally dangerous insecticides. This has resulted in a worldwide effort by wheat breeders to identify sources of genetic resistance to the Russian wheat aphid (Du Toit 1989 a).

Du Toit (1987) first reported genetic resistance to the Russian wheat aphid in South Africa in 1986 after screening wheat genotypes from native countries of the aphid *D. noxia* resistance in SA 1684 (PI 1377739) and SA 2199 (PI 262660) was found to be conferred by single dominant resistant genes, designated *Dn1* and *Dn2* (Du Toit 1989 b). A recessive resistance gene present in *Triticum tauschii* line SQ24 was subsequently designated *Dn3* (Nkongolo et al. 1991 a), while two non-allelic dominant resistance genes found in PI 372129 and PI 294994 were designated *Dn4* and *Dn5* by

Nkongolo et al. (1991 b) and Marais and Du Toit (1993), respectively.

The identification of *D. noxia* resistance genes has led to an ongoing breeding program in South Africa with the aim to identify and transfer *D. noxia* resistance genes to agriculturally important wheat cultivars. This program is based on the selection of resistant wheat plants after infestation with the aphid. This phenotype-based selection process is time-consuming and often not very accurate, which limits the rate at which resistance genes can be transferred to susceptible wheat cultivars (Du Toit 1987).

DNA-based molecular markers, such as restriction fragment length polymorphisms (RFLPs) (Botstein et al. 1980) and random amplified polymorphic DNAs (RAPDs) (Welsh and McClelland 1990; Williams et al. 1990), can be used for marker-assisted selection (MAS) (Melchinger 1990) of disease resistance in wheat (Schachermayr 1994, 1995). Devos and Gale (1992) predicted that RAPD analysis will be of limited use for linkage mapping in the complex hexaploid wheat genome, but proposed that RAPD markers may be useful for the characterization of introgressed chromosome segments. A number of introgressed resistance genes have subsequently been tagged in hexaploid wheat by means of RAPD analysis. These include the *Lr9* and *Lr24* leaf rust resistance genes (Schachermayr et al. 1994, 1995), the *Pm21* powdery mildew resistance gene (Hartl et al. 1993; Qi et al. 1996), the *Bt-10* common bunt resistance gene (Demeke et al. 1996), and the *Wsm1* wheat streak mosaic virus resistance gene (Talbert et al. 1996).

The aim of the present study was to identify RAPD markers linked to the *Dn2* Russian wheat aphid resistance gene. The identification of tightly linked molecular markers for this gene will allow the use of MAS for resistance breeding and will accelerate the development of new resistant cultivars. In addition, the identification of molecular markers for different *Dn* genes will assist in detecting *Dn* gene combinations in segregating populations and will make 'gene pyramiding' through MAS a viable prospect.

Materials and methods

Plant material

The wheat lines and the F_2 population used in this study were developed at the Small Grain Institute, Bethlehem, South Africa. The near-isogenic line 'Palmiet'*Dn2* (SA 2199/6* 'Palmiet') was developed by crossing the *Dn2* source-line SA 2199 six times to the susceptible South African spring cultivar 'Palmiet'. The final backcross line was selfed twice to produce the homozygous resistant line 'Palmiet'*Dn2*. One row of ten plants of each wheat line was grown under greenhouse conditions as described by Du Toit (1988). A bulked DNA sample, consisting of equal amounts of DNA from each of the ten individual plants, was used as a representative DNA sample for each wheat line.

The 90-plant F_2 population used for segregation analysis was developed using the original source parent SA 2199 and the susceptible parent 'Palmiet'. For segregation analysis, two 10-cm leaf segments were harvested from the primary leaves of each F_2 plant at the four-leaf stage and used for DNA isolation. After a recovery period of 3 days, the plants were infested with Russian wheat aphids and evaluated for the phenotypic segregation of resistance over a period of 21 days. A 1–10 disease severity scale was used with values 1–6 scored as resistant and 7–10 scored as susceptible (Tolmay 1995).

The SCAR and RAPD markers obtained were tested on several other wheat cultivars and lines containing the *Dn1*, *Dn2*, *Dn4* and *Dn5* resistance genes (see Table 4). Additional susceptible cultivars included in the study are 'Tugela', 'Molopo', 'Betta', 'Gamtoos', 'Letaba' and 'Hugenoot'.

DNA extraction

DNA samples were prepared from 0.3 g leaf samples using a modified DNA isolation method (Edwards et al. 1991). After precipitation of the DNA in ice-cold 100% ethanol for 5 min, the DNA was scooped out using a sterile pipette tip and washed in 70% ethanol for another 5 min. The DNA was air-dried and dissolved in 200 μl of sterile water. The DNA concentration of each sample was determined spectrophotometrically and the samples were diluted to 2.5 ng/μl for use in RAPD analysis.

RAPD-PCR conditions

The 300 oligonucleotide primers used in this study were obtained from Operon Technologies, Almeda, Calif., USA (kits A–O). The optimal conditions for the RAPD-PCR reaction were determined using modified Taguchi optimization methods (Cobb and Clarkson 1994). RAPD-PCRs were performed in 25 μl reaction mixtures under an oil overlay in 96-well microtiter plates (BIOzym, The Netherlands). The reaction mixtures contained 7.5 ng of template DNA, 1 × amplification buffer [20 mM $(NH_4)_2SO_4$, 75 mM Tris-HCl pH 9.0, 0.1% (v/v) Tween], 300 μ M of dNTPs, 0.001% (m/v) gelatin, 3 mM of $MgCl_2$, 7 pmol of 10-mer primer, and 0.6 U of *Taq* DNA polymerase (Advanced Biotechnologies, UK). Temperature cycling was performed using a thermal cycler (Hybaid$_{TM}$ Omnigene model TR3CM220) programmed for an initial step of 4 min at 94°C, followed by 40 cycles of 30 s at 94°C, 45 s at 35°C and 90 s at 72°C with a 1 s increase in each cycle, and a final step of 5 min at 72°C. Amplification products were analyzed by electrophoresis on 2% agarose gels (Seakem LE, FMC, USA) at 3 V/cm in 1 × TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0).

Linkage analysis

RAPD markers that were polymorphic in the NILs and displayed recombination fractions of less than 25% in a 12-plant subset of the 90-plant population were further analyzed in the rest of the F_2 population using standard RAPD-PCR conditions. The linear order of the RAPD markers in the *Dn2* linkage group was determined using MAPMAKER (Lander et al. 1987). Recombination fractions were transformed to linkage distances in centiMorgans (cM) according to Kosambi (1944).

Southern analysis of RAPD markers

To determine the feasibility of converting the RAPD markers to SCARs, the RAPD markers were cloned, labelled and used for

Southern analysis of the RAPD amplification products produced during the segregation analysis. One clone of each marker was labelled with 3000 Ci/mmol [α-^{32}P]-dATP (Megaprime DNA labelling system, Amersham, UK) and used for Southern analysis of a small amount (2 µl) of separated RAPD products.

SCAR design and analysis

Two RAPD markers ($OPB10_{880c}$ and OPN_{1400r}) were converted to SCARs (Kesseli et al. 1992; Paran and Michelmore 1993). The cloned fragments were sequenced from both ends and the sequence data used to design 22-bp SCAR primers containing the original ten bases of the RAPD primers and the first 12 internal bases adjacent to the RAPD primers. The SCAR primers were synthesized by DNAgency (Malvern, Pa. USA).

SCAR amplification of wheat genomic DNA was performed in 25 µl reaction mixtures containing 20 ng of template DNA, 1 × amplification buffer [20 mM $(NH_4)_2SO_4$, 75 mM TrisHCl pH 9.0, 0.1% (v/v) Tween], 150 µM of dNTPs, 0.001% (m/v) gelatin, 2 mM of $MgCl_2$, 5 pmol of each 22-mer primer, and 1 U of *Taq* DNA polymerase (Advanced Biotechnologies, UK), overlaid with one drop of mineral oil. Temperature cycling was performed using a thermal cycler (Hybaid™ Omnigene model TR3CM220) programmed for an initial step of 3 min at 94°C, followed by 35 cycles of 20 s at 94°C, 30 s at 62°C and 45 s at 72°C with a 1 s increase in each cycle, and a final step of 5 min at 72°C. Agarose electrophoresis was performed as described for RAPD analysis.

Results

RAPD analysis of near-isogenic lines

RAPD analysis of the two NIL combinations with 300 oligonucleotide primers yielded an average of nine RAPD bands per primer ranging in size from 300 to 3000 bp. Approximately 2700 RAPD loci were evaluated for potential linkage to the *Dn2* locus. Polymorphic RAPD fragments that were not reproducible, or could not be scored unambiguously, were discarded. RAPD analysis of the NILs and the resistance-source parent yielded four putatively linked RAPD markers amplified by four different primers (Table 1, Figs. 1, 2).

Linkage analysis of RAPD markers

The mean disease severity value at 21 days after infestation was 8.32 ± 1.08 for susceptible F_2 plants, while the mean value for resistant F_2 plants was 4.1 ± 1.44. Unambiguous resistance phenotypes were obtained for all the F_2 plants. The *Dn2* gene and the four linked markers segregated in a monogenic, dominant 3:1 Mendelian fashion at a level of significance of 0.05 (Table 2). Two RAPD markers ($OPM9_{1600r}$ and $OPN1_{400r}$) were linked in repulsion phase and two markers ($OPB10_{880c}$ and $OPO11_{900c}$) were linked in coupling phase with the *Dn2* locus (Table 1). MAPMAKER (Lander et al. 1987) generated a linkage map with a total size of 4.4 cM (Fig. 3).

Table 1 Summary of RAPD markers linked to the Russian wheat aphid resistance gene *Dn2*

Marker[a]	Gene	Linkage phase	Recombination fraction (%)	Linkage distance (cM)[b]
$OPB10_{880c}$	*Dn2*	Coupling	3.3	3.3
$OPM9_{1600r}$	*Dn2*	Repulsion	3.3	3.3
$OPN1_{400r}$	*Dn2*	Repulsion	3.3	3.3
$OPO11_{900c}$	*Dn2*	Coupling	4.4	4.4

[a] Subscripts indicate the linkage phase of the markers. c – coupling phase, r – repulsion phase
[b] Estimated from recombination fractions according to Kosambi (1944)

The repulsion-phase markers were expected to be present in the heterozygous resistant F_2 plants due to the dominant nature of RAPD markers and the presence of the 'susceptibility' allele. However, when tested in the F_2 population, the repulsion-phase markers were only present in the susceptible (homozygous recessive) F_2 individuals (Fig. 2).

Southern analysis of RAPD markers

Southern analysis of the RAPD products generated during segregation analysis (Fig. 1 B) confirmed that the cloned DNA fragments were the same as those that segregated in the F_2 population. No amplification products were detected in any of the lanes where the RAPD markers were previously scored as absent, even after prolonged exposure of the X-ray films. This corroborated the unexpected absence of the repulsion-phase markers from heterozygous resistant plants (data not shown).

Conversion of RAPD markers to SCARs

Sequence data obtained from the cloned fragments revealed that the amplified fragments were bordered by the original ten bases of the RAPD primers OPB10 and OPN1. Specific 22-mer SCAR primers were synthesized and named $B10_{880}$-1 and $B10_{880}$-2, and $N1_{400}$-1 and $N1_{400}$-2 (Table 3). The SCAR primers produced the same polymorphisms as the original RAPD markers, i.e. a 400-bp band (SCAR-$N1_{400r}$) and an 880-bp band (SCAR-$B10_{880c}$) that differentiated between resistant and susceptible NILs (Figs 4 A, 5 A). No amplification of the 880-bp band was observed in the susceptible F_2 plants (Fig. 4 A) or in the other susceptible cultivars 'Tugela', 'Molopo', 'Hugenoot' and 'Letaba' (Fig. 4 b, Table 4). The SCAR band was also absent in 'Palmiet' lines carrying other *Dn* genes, for example 'Palmiet' *Dn1* and 'Palmiet' *Dn5*. On the other hand, amplification with SCAR-$N1_{400}$ produced faint

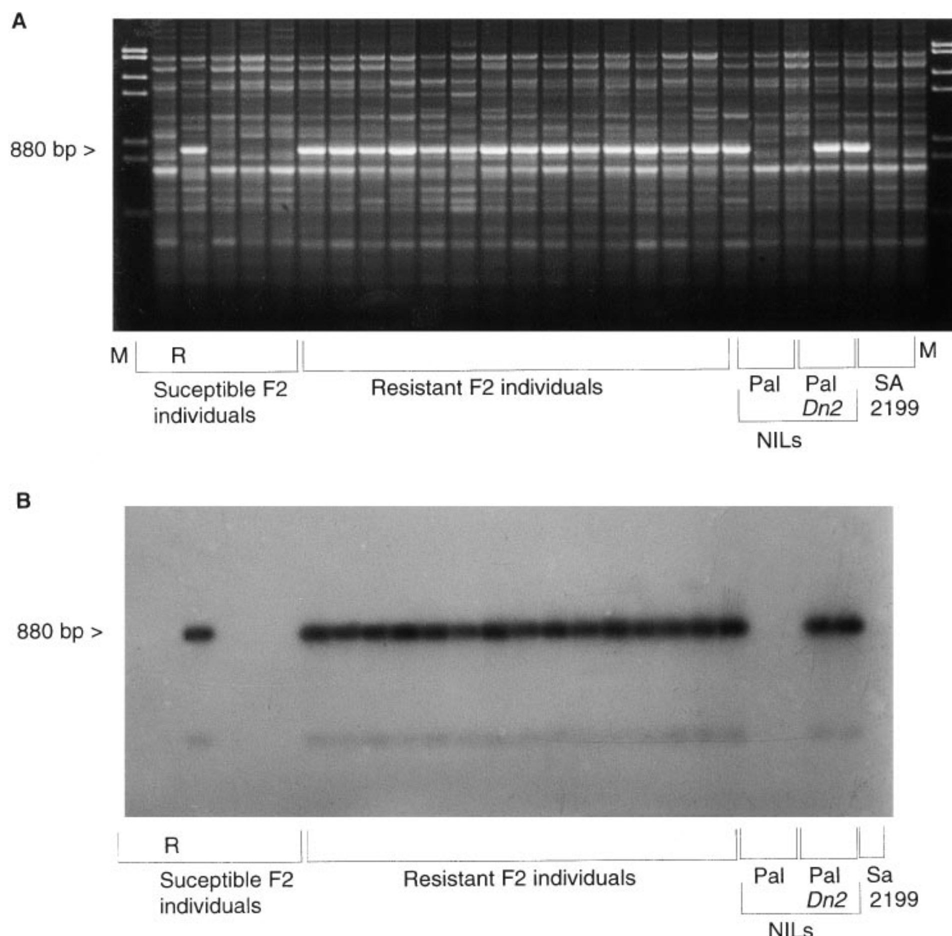

Fig. 1 A, B Segregation analysis of the coupling-phase RAPD marker $OPB10_{880c}$ linked to the *Dn2* resistance gene. The marker was identified by RAPD analysis of the susceptible cultivar 'Palmiet' (Pal) and the resistant near-isogenic line Palmiet *Dn2* (SA 2199/6* 'Palmiet'). *SA 2199* is the resistance source parent used to construct the F_2 population. (**A**) Segregation of the marker in 20 of the 90 F_2 individuals. (**B**) Southern analysis of the original RAPD products shown in (**A**) using the cloned RAPD marker as a labelled probe. *R* = recombinant F_2 individual. *M* is the molecular-weight marker III (λDNA digested with restriction enzymes *Hin*dIII and *Eco*RI)

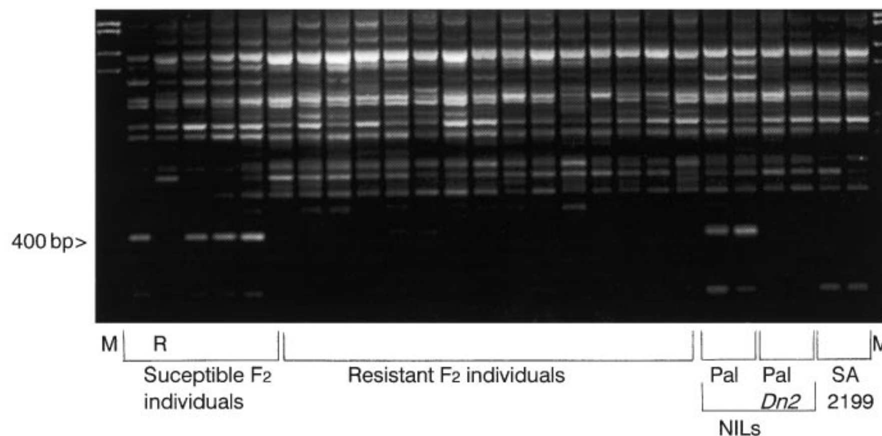

Fig. 2 Segregation of the repulsion-phase RAPD marker $OPN1_{400r}$ linked to the *Dn2* resistance gene in 20 of the 90 F_2 individuals. The marker was identified by RAPD analysis of the susceptible cultivar 'Palmiet' (Pal) and the resistant near-isogenic line Palmiet *Dn2* (SA 2199/6* 'Palmiet'). *SA2199* is the resistance source parent used to construct the F_2 population. R = recombinant F_2 individual. M is the molecular-weight marker III (λDNA digested with restriction enzymes *Hin*dIII and *Eco*RI)

amplification products in two resistant F_2 plants that were not previously amplified by the RAPD primers (Fig. 5 A).

SCAR-$B10_{880c}$ and SCAR-$N1_{400r}$ were also tested in other resistant genetic backgrounds. SCAR-$B10_{880c}$ was present in all the genetic backgrounds containing the *Dn2* resistance gene and absent in all the backgrounds with other *Dn* resistance genes, except in the *Dn4* containing source 'Corwa' (Quick et al. 1996) (Fig. 4 B, Table 4). SCAR-$N1_{400r}$ was generally only present in the susceptible cultivars. The marker detected the absence of *Dn2* in only two resistant lines, both containing the 'Hugenoot' susceptible background (Fig. 5 B, Table 4).

Table 2 Observed phenotypic and genotypic segregation ratios and chi-square values for goodness-of-fit to expected ratios for the F_2 progeny of a cross of SA 2199 and 'Palmiet'

Phenotype/genotype[a]	Number of plants		Expected ratio	Chi-square	P[b]
	Resistant/present	Susceptible/absent			
F_2 phenotype	72	18	3:1	0.95	0.33
$OPB10_{880c}$	75	15	3:1	2.90	0.09
$OPM9_{1600r}$	15	75	3:1	2.90	0.09
$OPN1_{400r}$	15	75	3:1	2.90	0.09
$OPO11_{900c}$	74	16	3:1	2.13	0.14

[a] Subscripts indicate the linkage phase of the markers. c – coupling phase, r – repulsion phase
[b] A value smaller than 0.05 indicates a significant deviation from goodness-of-fit

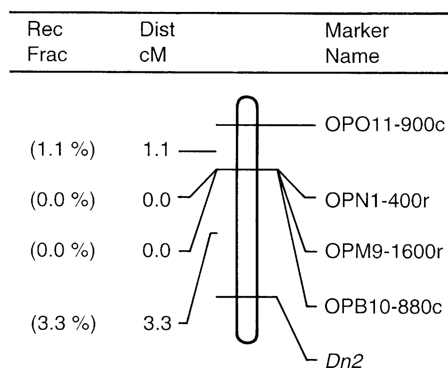

Fig. 3 Linear order of RAPD markers on the linkage map generated by MAPMAKER (Lander 1987). Linkage distances were calculated according to Kosambi (1944). *c* – coupling-phase marker, *r* – repulsion phase marker

Discussion

RAPD analysis of NILs has proved to be an effective approach towards the identification of closely linked molecular markers for important traits in wheat (Schachermayr et al. 1994, 1995; Demeke et al. 1996). In the present study, the analysis of 2700 RAPD loci in a set of NILs yielded four markers closely linked to the *Dn2* Russian wheat aphid resistance locus.

Martin et al. (1991) showed that the probability of finding a marker within a specified distance from a target gene depends on the size of the genome, the number of primers screened, and the degree of sequence divergence between the two near-isogenic lines in the region of the introgressed chromosome segment carrying the target gene. Using the formula proposed by Martin et al. (1991) and assuming random distribution of marker loci, the expected minimum distance between the resistance genes and any of the 2700 RAPD loci we scored is predicted to be 1.99 cM (at the 95% confidence level). This correlates with the minimum linkage distance of 3.3 cM observed in this study.

The coupling-phase marker $OPB10_{880c}$ was present in the resistant NIL ('Palmiet' *Dn2*) but absent in both its parents ('Palmiet' and SA 2199). $OPB10_{880c}$ seems to be a non-parental band co-segregating with *Dn2* resistance in the F_2 population. A possible explanation for the presence of this non-parental band in the F_2 progeny is the formation of heterodimers (Davis et al. 1995) in which one half of the heterodimer is contributed by the susceptible parent and the other half is situated on the linked introgressed chromosome segment. This could explain the absence of $OPB10_{880c}$ in SA2199, the presence of $OPB10_{880c}$ in the F_2 progeny and in Palmiet *Dn2,* and its observed linkage to *Dn2*.

The absence of the repulsion-phase markers in the heterozygous resistant F_2 individuals suggests the inability of the RAPD primers to prime at their target loci in the presence of the respective resistance alleles, i.e. possible template competition effects. This phenomenon has also been observed for all the repulsion-phase markers linked to other *Dn* genes (unpublished results). This seems completely contradictory to the dominant nature of RAPD markers and we are currently investigating this unexpected observation.

RAPD analysis is intrinsically less repeatable than specific PCR applications. To overcome this problem,

Table 3 Summary of SCAR primers derived from RAPD markers linked to the Russian wheat aphid resistance gene *Dn2*

SCAR primer	RAPD marker	Sequence[a]
$B10_{880}$-1	$OPB10_{880c}$	5′-CTGCTGGGACGAAGCGTTTGAC-3′
$B10_{880}$-2	$OPB10_{880c}$	5′-CTGCTGGGACCCGATGAATTGT-3′
$N1_{400}$-1	$OPN1_{400r}$	5′-CTCACGTTGGGAGCCATTGACG-3′
$N1_{400}$-2	$OPN1_{400r}$	5′-CTCACGTTGGCATCAGGGATAA-3′

[a] Underlined sequences represent the original RAPD marker

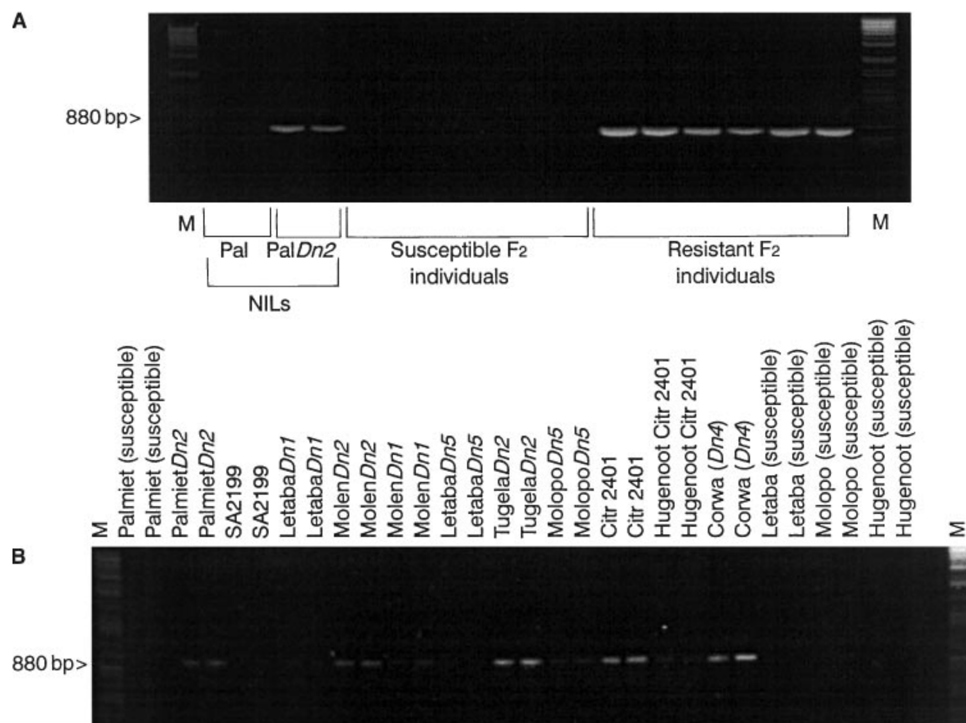

Fig. 4A, B SCAR primer $OPB10_{880c}$ used to screen: (**A**) 12 F_2 individuals from a *Dn2* segregating population, 'Palmiet' (Pal) and the resistant near-isogenic line Palmiet *Dn2* (SA 2199/6* 'Palmiet'); and (**B**) resistant and susceptible cultivars and lines from different genetic backgrounds. *M* is the molecular-weight marker III (λDNA digested with restriction enzymes *Hin*dIII and *Eco*RI).

Table 4 Presence (+) and absence (−) of the *Dn2* resistance gene and SCARs $B10_{880c}$ and $N1_{400r}$ in different South African wheat cultivars

Cultivar[a]	*Dn2* allele	SCAR markers	
		$B10_{880c}$	$N1_{400r}$
SA 1684 (*Dn1* resistance source parent)	−	−	−
SA 2199 (*Dn2* resistance source parent)	+	−	−
SA 463 (*Dn5* resistance source parent)	−	−	−
'Corwa' (*Dn4* resistance gene)	(?)	+	−
'Palmiet' *Dn2* (SA 2199/*6 'Palmiet')	+	+	−
'Palmiet' *Dn1* (SA 1684/*6 'Palmiet')	−	−	−
'Palmiet' *Dn5* (SA 463/*6 'Palmiet')	−	−	−
'Tugela' *Dn2* (SA 2199/*5 'Tugela')	+	+	−
'Molopo' *Dn5* (SA 463/5* 'Molopo')	−	−	−
'Letaba' *Dn1* (SA 1684/*5 'Letaba')	−	−	−
'Letaba' *Dn5* (SA 463/*5 'Letaba')	−	−	−
'Molen' *Dn2* (SA 2199/*5 'Molen')	+	+	−
'Molen' *Dn5* (SA 1684/*5 'Molen')	−	−	−
'Hugenoot' *Dn5* (SA 463/*4 'Hugenoot')	−	−	+
'Palmiet'[b]	−	−	+
'Tugela'[b]	−	−	+
'Molopo'[b]	−	−	+
'Gamtoos'[b]	−	−	+
'Letaba'[b]	−	−	+
'Hugenoot'[b]	−	−	+
Citr 2401[c]	(?)	+	−
(Citr 2401/*2 'Hugenoot')[d]	−	−	+

[a] All plant material was obtained from the Small Grain Institute, Bethlehem, South Africa
[b] All cultivars are susceptible to the Russian wheat aphid
[c] Cultivar with tested resistance to Russian wheat aphid but with an unidentified gene
[d] Cross with unverified Russian aphid resistance

we successfully converted two RAPD markers, $OPB10_{880c}$ and $OPN1_{400r}$, to SCAR markers, which are based on longer, more specific, PCR primers. Hybridization profiles obtained by probing the original segregating RAPD products with the cloned fragments confirmed the homology of the RAPD markers, the absence of background amplification, and the unambiguous nature of the RAPD polymorphisms.

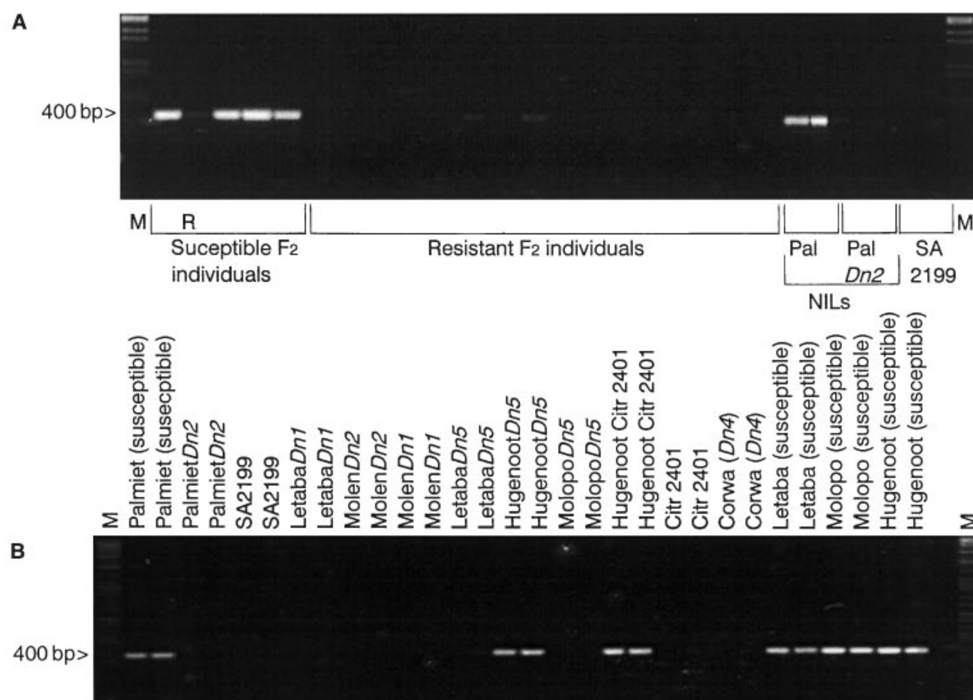

Fig. 5A, B SCAR primer $OPN1_{400r}$ used to screen: (**A**) 20 F_2 individuals from a *Dn2* segregating population, 'Palmiet' (Pal), the resistant near-isogenic line Palmiet *Dn2* (SA 2199/6* 'Palmiet') and *SA 2199* the resistance source parent used to construct the F_2 population. *R* – recombinant F_2 individual; and (**B**) resistant and susceptible cultivars and lines from different genetic backgrounds. M is the molecular-weight marker III (λDNA digested with restriction enzymes *Hin*dIII and *Eco*RI)

Both sets of SCAR primers produced single amplification products with the same molecular size and segregation phase as the original RAPD markers. Preliminary results indicate that SCAR-$B10_{880c}$ will be useful for selection of the *Dn2* gene in a variety of genetic backgrounds. The presence of the SCAR-$B10_{880c}$ in 'Corwa' (*Dn4* background) suggests either recombination of the marker and the resistance locus in Corwa, or else close association (allelism or tight linkage) of the *Dn2* and *Dn4* resistance genes in 'Corwa'. This observation is corroborated by the absence of the repulsion-phase marker, SCAR-$N1_{400r}$, in 'Corwa'.

The repulsion-phase marker SCAR-$N1_{400r}$ detected the absence of the *Dn2* gene in all the susceptible cultivars tested (Table 4). However, the marker failed to detect the absence of the *Dn2* gene in SA 1684 (*Dn1* source) and SA 463 (*Dn5* source) and most of the *Dn1*- and *Dn5*-containing isogenic lines. The absence of SCAR-$N1_{400r}$ from SA 1684 and SA 463 can be ascribed to background specificity. These sources are not related to the susceptible South African cultivars and differ therefore from these cultivars with respect to the marker allele. The failure of SCAR-$N1_{400r}$ to detect the absence of the *Dn2* gene in the *Dn1* and *Dn5* isogenic lines contradicts the absence of the coupling-phase marker SCAR-$B10_{880c}$ from these lines (Table 4). We believe that this unexpected behavior of SCAR-$N1_{400r}$ may be the result of the same unknown mechanism involved the absence of the marker (and the repulsion-phase RAPD markers) from the heterozygous F_2 plants.

The identification of RAPD markers linked to the *Dn2* resistance gene and the conversion of these markers to SCARs will allow routine MAS for this gene in wheat breeding programs. MAS with tightly linked molecular markers is not only accurate and fast, but will alleviate the use and maintenance of large aphid stocks needed for phenotype-based selection procedures. Identification of RAPD markers and SCARs for other *Dn* genes will allow breeders to select for these resistance genes in genetic backgrounds containing more than one *Dn* gene. This will create the unique opportunity for using MAS to combine (or pyramid) two or more *Dn* genes in a single wheat line.

The occurrence of different biotypes with different reactions to plant resistance genes has been reported for a number of aphid species (Tyler et al. 1987). The possibility therefore exists that the release of new *D. noxia*-resistant wheat cultivars could lead to the development of Russian wheat aphid biotypes which can overcome the effect of single dominant resistance genes. Marker-assisted pyramiding of *D. noxia* resistance genes to produce wheat lines with complex and durable resistance will serve, not only to prevent the development of new biotypes, but also to increase the current levels of Russian wheat aphid resistance in South African wheat cultivars.

Acknowledgements This work was financially supported by the University of the Orange Free State, Small Grain Institute and the Foundation for Research Development. Genetic material was provided by Dr. H. A. van Niekerk, Small Grain Institute, Bethlehem, South Africa.

References

Botstein D, White RL, Skolnik M, Davies RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Brooks L, Hein G, Johnson D, Legg G, Massey B, Morrison P, Weiss M, Peairs FB (1994) Economic impact of the Russian wheat aphid in the western United States: 1991–1992. Great Plains Agric Council Pub 147:250–268

Cobb DB, Clarkson JM (1994) A simple procedure for optimizing the polymerase chain reaction (PCR) using modified Taguchi methods. Nucleic Acids Res 22:3801–3805

Davis TM, Yu H, Haigis K-M, McGowan PJ (1995) Template mixing: a method of enhancing detection and interpretation of co-dominant RAPD markers. Theor Appl Genet 91:582–588

Demeke T, Laroche A, Gaudet DA (1996) A DNA marker for the common bunt resistance gene in wheat. Genome 39:51–55

Devos KM, Gale MD (1992) The use of random amplified polymorphic DNA markers in wheat. Theor Appl Genet 84:567–572

Du Toit F (1987) Resistance in wheat (*Triticum aestivum*) to *Diuraphis noxia* (Hemiptera: Aphididae). Cereal Res Commun 15:175–179

Du Toit F (1988) A greenhouse test for screening wheat seedlings for resistance to the Russian wheat aphid, *Diuraphis noxia* (Homoptera: Aphididae). Phytophylactica 20:321–322

Du Toit F (1989 a) Inheritance of resistance in two *Triticum aestivum* lines to Russian wheat aphid (Homoptera: Aphididae). J Econ Entomol 82:1251–1253

Du Toit F (1989 b) Components of resistance in three bread wheat lines to *Diuraphis noxia* (Mordvilko). J Econ Entomol 82:1779–1781

Edwards K, Johnstone C, Thompson C (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res 19:1349

Gilchrist LI, Rodriquez R, Burnett PA (1984) The extent of Freestate streak and *Diuraphis noxia* in Mexico. In: Burnett PA (ed) Proc Barley Yellow Dwarf Wrkshp, CIMMYT. El Bátan, Mexico, pp 157–163

Hartl L, Weiss H, Zeller FJ, Jahoor A (1993) Use of RFLP markers for the identification of alleles of the *Pm3* locus conferring powdery mildew resistance in wheat (*Triticum aestivum* L.) Theor Appl Genet 86:959–963

Hewitt PH, Van Niekerk GJJ, Walters MC, Kriel CF, Fouche A (1984) Aspects of the ecology of the Russian wheat aphid, *Diuraphis noxia*, in the Bloemfontein district. I. The colonization and infestation of sown wheat, identification of summer hosts and cause of infestation symptoms. Tech Commun, Department of Agriculture, Republic of South Africa 191:3–13

Kesseli RV, Paran I, Michelmore RW (1992) Efficient mapping of specifically targeted genomic regions and the tagging of these regions with reliable PCR-based genetic markers. Crop Sci Soc Am – Am Soc Hort Sci – Am Genet Assn Joint Plant Breed Symp Ser, Minneaplois, Crop Sci Soc Am, Madison, Wisconsin, pp 31–36

Kosambi DD (1944) The estimation of map distances from recombination values Ann Eugen 12:172–175

Lander GS, Groon P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburk L (1987) MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Marais GF, Du Toit F (1993) A monosomic analysis of Russian wheat aphid resistance in the common wheat PI 294994. Plant Breed 111:246–248

Martin GB, Williams JKG, Tanksley SD (1991) Rapid identification of markers linked to the *Pseudomonas* resistance gene in tomato by using random primers and near-isogenic lines. Proc Natl Acad Sci USA 88:2336–2340

Melchinger AE (1990) Use of molecular markers in breeding for oligogenic disease resistance. Plant Breed 104:1–19

Morrison WP (1988) Current distribution and economic impact. In: Peairs FB, Pilcher SD (eds) Proc 2nd Russian Wheat Aphid Wkshp. Denver, Colarado, p 5

Nkongolo KK, Quick JS, Limin AE, Fowler DB (1991 a) Sources and inheritance of resistance to the Russian wheat aphid in *Triticum* species, amphiploids and *Triticum tauschii*. Can J Plant Sci 71:703–708

Nkongolo K.K, Quick, JS, Peairs FB, Meyer WL (1991 b) Inheritance of resistance of PI 372129 wheat to the Russian wheat aphid. Crop Sci 31:905–907

Paran I, Michelmore RW (1993) Development of reliable PCR-based markers linked to downey mildew resistance genes in lettuce. Theor Appl Genet 85:985–993

Qi L, Cao M, Chen P, Li W, Liu D (1996) Identification, mapping and application of polymorphic DNA associated with resistance gene *Pm21* of wheat. Genome 39:191–197

Quick JS, Nkongolo KK, Peairs FB, Rudolph JB (1996) Registration of Russian wheat aphid-resistant wheat germplasm CORWA1. Crop Sci 36:217

Schachermayr G, Siedler H, Gale MD, Winzeler H, Winzeler M, Keller B (1994) Identification and localization of molecular markers linked to the *Lr9* leaf rust gene of wheat. Theor Appl Genet 88:110–115

Schachermayr G, Messmer MM, Feuillet C, Winzeler H, Winzeler M, Keller B (1995) Identification of molecular markers linked to the *Agropyron elongatum*-derived leaf rust resistance gene *Lr24* in wheat. Theor Appl Genet 90:982–990

Talbert LE, Bruckner PL, Smith LY, Sears R, Martin TJ (1996) Development of PCR markers linked to resistance to wheat streak mosaic virus in wheat. Theor Appl Genet 93:463–467

Tolmay VL (1995) The inheritance of Russian wheat aphid (*Diuraphis noxia*) resistance in two *Triticum aestivum* lines. MSc thesis, University of the Orange Free State, South Africa

Tyler JM, Webster JA, Merkle OG (1987) Designations for genes in wheat germplasm conferring greenbug resistance. Crop Sci 27:526–527

Webster JA, Starks KJ, Burton RL (1987) Plant resistance studies with *Diuraphis noxia* (Mordvilko) (Homoptera: Aphididae), a new United States wheat pest. J Econ Entomol 80:944–949

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213–7218

Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535