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Development of markers linked to *Diuraphis noxia* resistance in wheat using a novel PCR-RFLP approach

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Abstracts Through random amplified polymorphic DNA (RAPD) analysis we identified a putative marker linked to the *Dn5* resistance gene. This marker was converted to a more reliable sequence-characterised-amplified regions (SCAR) marker. The initial SCAR marker amplified the correct amplification product but failed to discern between the susceptible and resistant individuals. Hence, it was utilised to sequence the internal fragment. All nested primers designed from the internal sequences were also unable to produce any polymorphism between the susceptible and resistant cultivars. Restriction digests were then performed on these fragments, and the restriction enzyme *Eco*RI was able to discern between the susceptible and resistant F_2 individuals of the *Dn5* population. This granted one marker amplified with the internal SCAR primer set $OPF14_{1083}$ the ability to differentiate between parental individuals carrying the *Dn5* genes. This marker was tested in a segregating F_2 population carrying the *Dn5* resistance gene and proved able to differentiate between the segregating individuals. This marker may prove useful in marker assisted selection (MAS), although performing restriction digests may hamper the throughput of a high number of samples.

Key words Russian wheat aphid · Near-isogenic lines · Restriction digests · RAPD · SCAR

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Introduction

Since the beginning of time grasses have fed the world's populations. Today grasses, rice, wheat and some five other cereals are the major source of nutrition for more than 90% of the world's population (Moore et al. 1993). However, several pests threaten the cultivation of these crops. One of the most destructive pests found on wheat (*Triticum aestivum* L.) is the Russian wheat aphid (*Diuraphis noxia* Mordvilko). This pest has become a major economic nuisance in almost all wheat-producing countries (Dong and Quick 1995; Nkongolo et al. 1991a, b). The Russian wheat aphid has also been a hampering factor in the production of winter wheat in South Africa (Du Toit 1992). It has been estimated that this pest can cause between a 35% and 60% loss in crop yield after infestation has occurred, resulting in major economic losses (Kindler et al. 1993; Nkongolo et al. 1991a, b).

To date, insecticides have mostly been used to combat the destructiveness of the Russian wheat aphid (Du Toit 1986; Nkongolo et al. 1990). However, the curling of the leaves and droughts are making it increasingly difficult to use insecticides as a control measure of these aphids (Du Toit 1992). The excessive use of chemicals is not an economical or environmental feasible approach, and all efforts should be taken to avoid this (Dong and Quick 1995; Du Toit 1986). Staggering amounts of money have been spent on chemical control of the Russian wheat aphid in certain parts of South Africa and other regions of the world. This will hamper the production of wheat in future (Du Toit 1988).

In 1984, plant breeders began to look for resistance to Russian wheat aphid in certain cultivars to stem the excessive use of chemical application (Dong and Quick 1995; Du Toit 1989). These resistant cultivars originate mostly from the countries in which the Russian wheat aphid is endemic, namely the former Soviet Union, Iran, Bulgaria and the Middle East (Du Toit 1992; Zemetra et al. 1990).

The use of host plant resistance towards this pest is the most effective control measure to date. It is also an

Table 1 Near-isogenic lines and F_2 populations used in this study

Cultivar/population	Description	Origin
Palmiet	Susceptible (recurrent) parent	SGI[a], South Africa
SA 463 (PI 294994)	*Dn5* resistance source parent	Bulgaria
Palmiet *Dn5*	SA 463/5* Palmiet	SGI[a], South Africa
Dn5 population	Palmiet ×SA 463 –F_2	SGI[a], South Africa

[a] SGI, Small Grain Institute, Bethlehem, South Africa

economically and environmentally sound approach (Du Toit 1992). However, coupled with the use of resistant cultivars in breeding programmes is the need to be able to quickly identify the cultivars that carry the gene of interest in order to provide a better product. This procedure has been greatly expediated with the recent advances in molecular biology (Weeden et al. 1994); in particular for plant breeders, in molecular marker technology. Molecular markers have been used as a tool in fingerprinting exercises, phylogenetic studies, gene tagging, genome mapping and identifying similarities among inbreds (Yu et al. 1993).

The selection for resistance genes on a phenotypic scale is time-consuming and can lead to misclassification (Procunier et al. 1997). It is therefore an appropriate approach to search for molecular markers linked to Russian wheat aphid resistance. The development of molecular markers linked to a trait of interest is useful in that it enables the breeder to detect the presence of a gene without waiting for its phenotypic expression. This shortens the time necessary to incorporate the gene of interest into a new cultivar and make it possible to pyramid several genes (Feuillet et al. 1995).

Although, several techniques have been used to identify markers linked to disease resistance in wheat, the most documented ones are random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) (Demeke et al. 1996; Devos and Gale 1992; Schachermayr et al. 1994). RAPD is a commonly used tool to identify new markers linked to different genes of interest, it has several distinct advantages over some other techniques in that it is easy to perform and fast. However, it has a tremendous drawback in its lack of reproducibility within and between laboratories. It is therefore necessary to convert these markers into more usable markers. Conversion of RAPD markers to sequence characterised amplified regions (SCAR) markers is a feasible method to enhance the reliability of the identified markers. To date, ten putative RAPD markers linked to Russian wheat aphid resistance, with linkage from 43.7 cM (*Dn1*) to 4.4 cM (*Dn2*), have been reported (Venter et al. 1998). Four of these markers are closely linked to the *Dn2* gene, but only two, $OPB10_{880c}$ and $OPN1_{400r}$, have been converted to effective sequence characterised amplified regions (SCAR) markers (Myburg et al. 1998).

The aim of the study presented here was to convert RAPD markers linked to the *Dn5* resistance gene into markers that can be of use in marker-assisted selection (MAS).

Materials and methods

Wheat material

The Small Grain Institute, Bethlehem, South Africa developed the plant material used in this study (Table 1). The near-isogenic line (NIL) was developed by backcrossing the *Dn5* source line (SA 463) to the susceptible Palmiet spring cultivar. The plants were grown under greenhouse conditions (Du Toit 1988), and DNA was extracted from the two oldest leaves at the four-leaf stage. Crossing the original resistant parent with the susceptible Palmiet developed a separate F_2 population. This F_2 population segregated according to Mendelian fashion (Myburg et al. 1998). After removal of the oldest leaves for extraction, the plants were infested with aphids and evaluated for tolerance on a 1–10 phenotypic scale: 1 being the most resistant and 10 the most susceptible.

DNA extraction and RAPD analysis

DNA was extracted according to a modified extraction method of Edwards et al. (1991). Determination of the optimal RAPD-PCR (polymerase chain reaction) conditions was done using a modified Taguchi optimisation method (Cobb and Clarkson 1994). The RAPD reactions were performed in 96-well microtitre plates (BIOzym) as 25-µml volumes underneath an oil underlay. The reactions contained 7.5 ng template DNA, 1×amplification buffer [20 m*M* $(NH_4)SO_4$, 75 m*M* TRIS-HCl pH 9.0, 0.1% (v/v) Tween 20], 300 µm*M* dNTPs, 0.001% (m/v) gelatin, 3 m*M* $MgCl_2$, 7 pmol primer (OPF14) (Operon Technologies, Alameda, Calif.) and 0.6 U *Taq* DNA polymerase (Advanced Biotechnologies). The reactions were performed in a Hybaid™ Omnigene model TR3CM220 thermocycler. The reaction had an introductory denaturing step at 94°C for 4 min; followed by 40 cycles of 94°C for 30 s, 35°C for 45 s, 72°C for 90 s with a 1-s increase in elongation time after each cycle; the final step was 72°C for 5 min. The products were separated on 2% agarose gels (Seakem LE, FMC) at 3 V/cm in 1×TAE buffer (40 m*M* TRIS-acetate, 1 m*M* EDTA, pH 8.0) and visualised under UV light after staining with ethidium bromide (0.5 µmg/ml). All reactions were performed in duplicate and repeated over time and material.

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 F_2 population were further analysed using standard RAPD-PCR conditions. Linkage distances between the markers and the *Dn5* gene analysed were determined using MAPMAKER (Lander et al. 1987). The Kosambi function was used to convert the recombination frequencies to centiMorgan (Kosambi 1944).

Conversion to SCAR markers

Two RAPD markers ($OPB2_{946}$, $OPF14_{1083}$) were selected for conversion to SCAR markers (Table 2). These fragments were cloned with a pGEM-T cloning kit (Promega) according to the manufacturer's specifications. Sequencing of these fragments was performed on an ABI 377 sequencing platform (Applied Biosystems).

Table 2 Putative RAPD markers identified in the *Dn5* population (Venter et al. 1998)

[a] Estimated from recombination fractions according to Kosambi (1944)

Marker	Gene	Linkage phase	Recombination fraction (%)	Linkage distance (cM)[a]
$OPB2_{946}$	*Dn5*	Repulsion	7.7	7.7
$OPB2_{1020}$	*Dn5*	Repulsion	2.2	2.2
$OPF14_{1083}$	*Dn5*	Coupling	5.5	5.5

Designing SCAR primers from the first ten internal bases of the fragments did not result in discriminating primers. Therefore, the fragments were sequenced completely, after of nested primers had been designed to sequence through the central region of the fragments. The non-differentiating SCAR primers were used to sequence the fragments amplified from the susceptible cultivars. The two resulting sequences were compared and any base substitutions noted. Nested SCAR primers were designed around these substitutions.

SCAR amplification of wheat genomic DNA was performed in 25-µml reaction volumes underneath an oil overlay. The SCAR reactions contained 5 ng template DNA, 1×amplification buffer [20 m*M* $(NH_4)SO_4$, 75 m*M* TRIS-HCl pH 9.0, 0.1% (v/v) Tween 20], 150 µ*M* dNTPs, 0.001% gelatin, 2 m*M* $MgCl_2$, 5 pmol of each 22-mer primer, and 1 U Red Hot™ *Taq* DNA polymerase (Advanced Biotechnologies). Temperature cycling was performed in a Hybaid™ Omnigene model TR3CM220 thermocycler. The reaction had an introductory denaturing step at 94°C for 3 min, followed by 35 cycles of 94°C for 20 s, 62°C for 30 s, 72°C for 45 s with a 1-s increase in elongation time after each cycle, the final step was 72°C for 5 min. The products were separated as described for the RAPD analysis on 1% agarose (Seakem LE, FMC) and visualised as previously described.

Restriction enzyme digest of non-differentiating SCAR markers

SCAR amplification was performed as described in the previous section. For restriction analysis the following 12 enzymes were considered: *Alu*I, *Bam*HI, *Dde*I, *Dra*I, *Eco*RI, *Hind*III, *Mse*I, *Not*I, *Pst*I, *Sal*I, *Sma*I and *Xba*I. These enzymes were chosen from restriction data collected from Webcutter (http://www.medkem.gu.se/cutter/). The PCR products were divided into 18.5 µml aliquots, and 2 U of different restriction enzymes, chosen from the generated sequence data, and 0.5–1 µl of 2 M NaCl stock (depending on the buffer used by the different enzymes) were added (Blanck et al. 1997). The volume was then adjusted to 20 µl and incubated under an oil overlay at 37°C for 1–3 h, unless the manufacturers stipulated another temperature. The restriction digest products were separated on 2% agarose (Seakem LE, FMC) and visualised as previously described. All reactions were repeated over time and by another researcher.

Results

RAPD analysis

RAPD analysis of the NILs containing the *Dn5* gene with 300 10-mer arbitrary primers yielded, on average, nine scorable bands per primer. These bands ranged in size from 300 to 3000 bp. Only 12 primers failed to amplify fragments with the wheat genome as template. Polymorphic fragments that were not reproducible or scored unambiguously were not included in this study. Primers producing polymorphisms between the NIL accessions were evaluated for their ability to produce polymorphisms between the parental lines used to construct the NIL accessions. RAPD markers that were present in coupling or repulsion phase between the source lines and the NIL accessions were evaluated for linkage segregation in a 96-plant F_2 population. This elimination process yielded three putatively linked RAPD markers, one in coupling and two in repulsion phase. The RAPD marker amplified with the primer set OPF14 is linked in the couplings phase to the *Dn5* resistance gene at 5.5 cM and was chosen for further development as a SCAR marker (Fig. 1) (Venter et al. 1998). MAPMAKER (Lander et al. 1987) generated a linkage map with the sizes ranging from 7.7 cM to 2.2 cM (Fig. 2).

Conversion to SCAR and subsequent analysis of genomic DNA

Determination of the first few internal bases yielded enough information to develop allele-specific SCAR primers. Testing these primers on a segregating F_2 population showed that all the SCAR markers amplified the original fragments amplified by the RAPD primers. However, the SCAR markers amplified fragments in both the susceptible and resistant F_2 individuals. This created the need to sequence the whole of the SCAR fragments to determine if there is an exploitable amount of difference between the susceptible and resistant fragments amplified from the parental lines. We were able to identify a small number of base differences between the different sequences (not shown). These subtle differences amounted to single base insertions and substitutions. This was employed for design of internal primer sets that should be able to differentiate between the susceptible and resistant cultivars. However, all of the primers tested failed to achieve this (not shown). Therefore, it became necessary to do enzymatic digests of all the amplified fragments to uncover a marker that would be of use.

PCR-RFLP analysis of SCAR generated fragments

Restriction analysis of the different fragments yielded one polymorphism for the *Dn5* resistance gene (Fig. 3). *Eco*RI produced a profile with three prominent bands found in both the parents and the offspring. There was also a differentiating band between the parental lines at approximately 300 bp that was also detected in the susceptible individual found in the segregating F_2 popula-

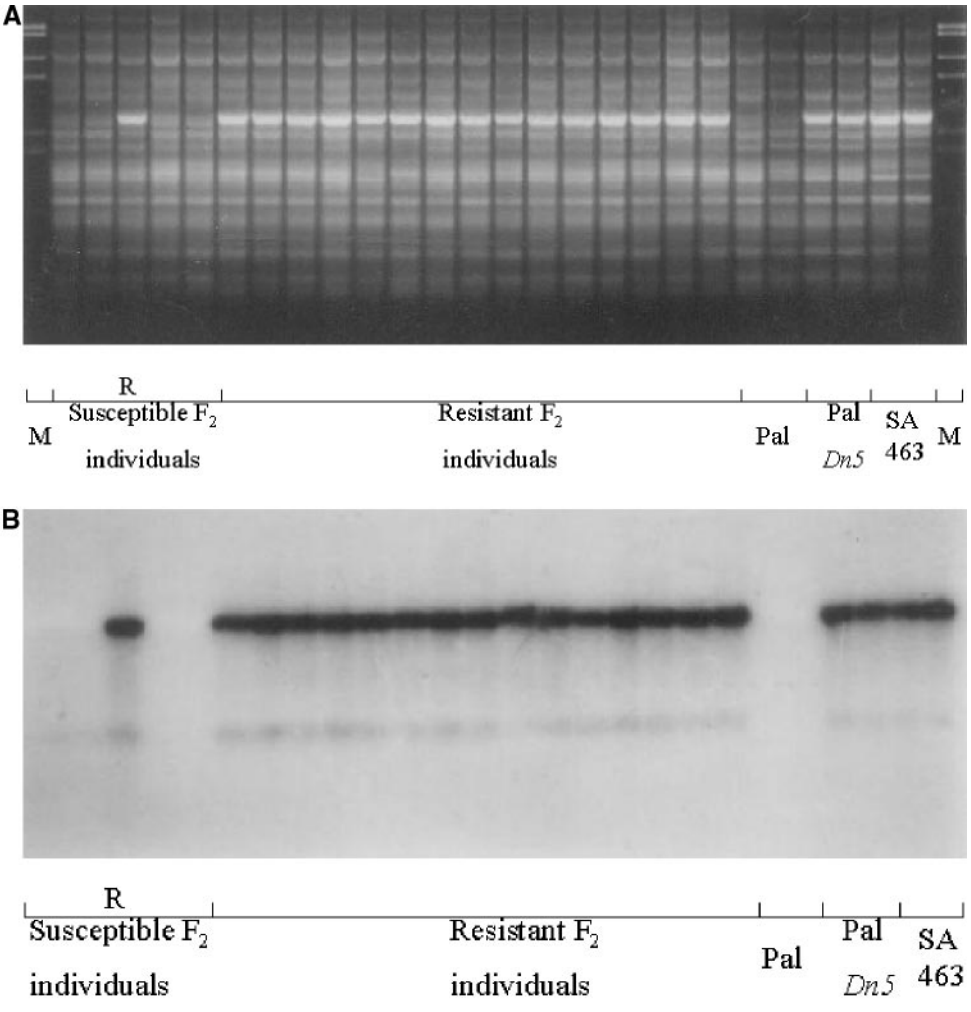

Fig. 1A, B Analysis of the coupling phase RAPD marker $OPF14_{1083}$ on a segregating F_2 population. This marker was identified through a RAPD analysis of the susceptible cultivar Palmiet and the resistant near-isogenic line Palmiet *Dn5* (SA 463/5*Palmiet). SA 463 is the resistant source parent used in the development of the resistant F_2 population. **A** Segregation of the coupling phase marker in 20 of the 90 F_2 individuals, **B** Southern analysis of the marker on the segregating RAPD products in **A** to verify if the marker truly segregates in population. R=Recombinant F_2 individual, *M*=molecular-weight marker III (λ DNA digested with *Eco*RI and *Hind*III)

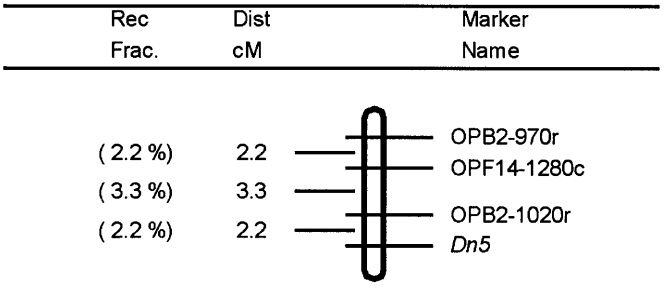

Fig. 2 Linkage map generated with MAPMAKER of the linear order of RAPD markers segregating in the *Dn5* F_2 population (Venter et al. 1998). All the linkage distances were calculated according to Kosambi (Kosambi 1944). *c*=Coupling phase marker, *r*=repulsion phase marker

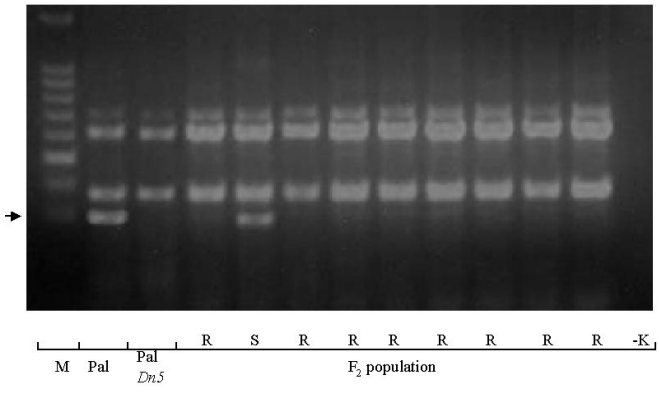

Fig. 3 PCR-RFLP analysis of the segregating coupling phase marker $OPF14_{1083}$. *Eco*RI restriction analysis of a subset of the segregating F_2 population. *Arrow* indicates the band able to differentiate between the susceptible (*S*) and resistant individuals (*R*). *M*=100 bp ladder as molecular-weight marker, K=Negative control

tion. This rendered the enzyme capable of differentiating between the susceptible Palmiet and resistant Palmiet *Dn5* parents as well as the individuals in a segregating F_2 population. All other enzymes failed to distinguish between the different parents.

Discussion

The inability to convert the RAPD markers to SCAR markers showed the same trend as found in several other studies where a high number of polymorphisms were

identified through RAPD analysis and only a small subset of these was convertible to SCAR markers (Adam-Blondon et al. 1994; Borovkova et al. 1997; Masuelli et al. 1995). This phenomenon is in accordance with the hypothesis that although it is theoretically easy to convert RAPD markers to SCAR markers, in practice this is not usually the case (Blake et al. 1996). When a polymorphism based on a short arbitrary primer is converted to a more specific and longer primer, the polymorphism is lost. This may be due to the loss of the uniqueness of the primer-binding site through the loss of the base substitution on which the polymorphism is based (Masuelli et al. 1995). It is also known that when a short 10-mer primer anneals to a long template, the middle bases pair more stringently to the template than to those on the 5′- and 3′-ends (Chen and Wu 1997). This results in the creation of a specific primer that contains two bases that are not complementary to the template, creating the possibility that spurious amplification can occur. Another hypothesis is due to the high level of repetitive DNA contained in cereal genomes. This causes the specific primer to amplify a homoeologous loci located on another chromosome which also gives rise to a similar banding pattern (Penner et al. 1996). The existence of homoeology between the three genomes of hexaploid wheat has been proven with aneuploid genetic stock (Devos and Gale 1997). It was found that certain chromosomes could substitute for each other, indicating that these chromosomes should carry the same genes. This again points towards the possibility that the more robust marker will be able to detect the different homoeologous loci.

We also found that the priming sites identified by the RAPD markers were not located in the ‘window’ that had been introgressed along with the resistance gene. This was evident from the SCAR marker amplifying fragments of equal length in both the susceptible and resistant cultivars. Sequence homology between these fragments is also high enough that they are clearly amplified from the same or very closely associated loci in the respective cultivars. Therefore, the occurrence of the original polymorphism was most likely caused by a base substitution, as earlier mentioned, or that a competition effect exists between the different genomes incorporated in hexaploid wheat. This competition would result in the differential amplification of certain parts of the wheat genome. A study conducted by Halldén et al. (1996) showed that when different fractions of an artificially created genome are amplified through RAPD, different fractions give rise to different banding patterns. This indicates that there is a definite correlation between competition effects and erroneous amplification with the RAPD technique.

Stam and Zeven (1981) stated that the distribution of the donor genome found in the NILs is not mirrored by the fraction of its DNA in the genome. This means that after a series of backcrosses the number of introgressed genes, other than the gene of interest, should be small. Through calculations and assuming that normal recombination take place, it has been shown that after five backcrosses (BC) the donor segment in the NILs comprises 22.8% of the NIL target chromosome. This would suggest that the size of the donor fragment on chromosome 7D in the BC_5 would be approximately 41 cM. If one considers that the distribution around the gene of interest is not symmetrical, it will explain why we were able to identify markers located up to 25 cM away from the resistance genes. Through further analysis by our group, it was shown that the polymorphism level detected by RAPDs between the donor and recurrent parent was about 7%. The recurrent susceptible parent Palmiet displayed even higher levels of genetic similarity to the donor parents (5.2%).

If we take the low amount of genetic dissimilarity between the different cultivars into consideration, it becomes possible that the original polymorphisms were based on a very low polymorphism rate. Once these polymorphisms were converted to more robust markers, their uniqueness was lost, and they once again amplified a fragment located at the same locus in both the susceptible and resistant NILs. This seems to be the most plausible explanation for the failure of the SCAR primers to differentiate between the different parents and the individuals in the F_2 population. As the SCAR markers failed to discern between the susceptible and resistant individuals, with the presence and absence of an amplified product, it rendered the simplification of the detection system useless (Kindler et al. 1993).

Southern analyses of all RAPD-generated fragments were carried out, and all the fragments segregated in accordance with the RAPD profiles (Fig. 1). The only unexpected development was that the genomic hybridisations displayed low signal, low-copy number characteristics which were in contrast with the high-copy number hybridisation profiles expected from RAPD fragments that contain repetitive DNA.

Amplification of the susceptible and resistant cultivars yielded only one possible restriction site linked to the *Dn5* gene that can be used, out of a total of twelve chosen enzymes. This PCR-RFLP has limited use in breeding programmes, as it is relatively cumbersome to use, not as cost effective and more time-consuming. However, it can be useful when a lower sample throughput is required. If a breeding facility holds the ability to use a combination of PCR and restriction digests of these products, they would be able to make extensive use of these types of markers. They could also be of use in map-based cloning as they are linked in the coupling phase and in the saturation of the wheat genomic map. For this reason the RAPD markers have been distributed to other groups to determine their mapping ability in different wheat cultivars. The results from these studies are as yet unavailable.

If the cost in developing SCAR markers, which necessitates the sequencing of the internal bases and the development of deeply nested primers, proves to be too high, a cheaper option is to use PCR-RFLP. It would be easier to test the efficiency and discrimination ability of different enzymes than to do the whole sequencing exercise. It would also be more easier to do this, as the initial

failure of our SCAR markers could not be rectified through intensive sequencing and primer design.

Although we were able to identify markers linked to Russian wheat aphid resistance in relative close proximity, we were unable to convert them to easy-to-use SCAR markers. The need existed to make use of PCR-RFLP to differentiate between the susceptible and resistant cultivars. These markers will be of use in MAS and will hopefully aid in development of resistant cultivars in relative shorter time-spans.

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References

Adam-Blondon AF, Sévignac M, Bannerot H, Dron M (1994) SCAR, RAPD and RFLP markers linked to a dominant gene (*Are*) conferring resistance to anthracnose in common bean. Theor Appl Genet 88:865–870

Blake TK, Kadyrzhanova D, Shepherd KW, Islam AKMR, Langridge PL, McDonald CL, Erpelding J, Larson S, Blake NK, Talbert LE (1996) STS-PCR markers appropriate for wheat-barley introgression. Theor Appl Genet 93:826–832

Blanck A, Gluck B, Wartbichler R, Bender S, Pöll M, Brandl A (1997) Activity of restriction enzymes in a PCR mix. Biochemica 3:25

Borovkova IG, Jin Y, Steffenson BJ, Kilian A, Blake TK, Kleinhofs A (1997) Identification and mapping of a leaf rust resistance gene in barley line Q21861. Genome 40:236–241

Chen X, Wu R (1997) Direct amplification of unknown genes and fragments by uneven polymerase chain reaction. Gene 185: 195–199

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

Demeke T, Laroche A, Gaudet DA (1996) A DNA marker for the *Bt-10* 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

Devos K, Gale MD (1997) Comparative genetics in the grasses. Plant Mol Biol 35:3–15

Dong H, Quick JS (1995) Inheritance and allelism of resistance to the Russian wheat aphid in seven wheat lines. Euphytica 81:299–303

Du Toit F (1986) Economic thresholds for *Diuraphis noxia* (Hemiptera: Aphididae) on winter wheat in the eastern Orange Free State. Phytophylactica 18:107–109

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) Inheritance of resistance in two *Triticum aestivum* lines to Russian wheat aphid (Homoptera: Aphididae). J Econ Entmol 82:1251–1253

Du Toit F (1992) Russian wheat aphid resistance in a wheat line from the Caspian sea area. Cereal Res Commun 20:55–61

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

Feuillet C, Messmer M, Schachermayr G, Keller B (1995) Genetic and physical characterization of the *Lr1* leaf rust resistance locus in wheat (*Triticum aestivum* L.). Mol Gen Genet 248:553–562

Halldén C, Hansen M, Nilsson N-O, Hjerdin A (1996) Competition as a source of errors in RAPD analysis. Theor Appl Genet 93:1185–1192

Kindler SD, Jensen KB, Springer TL (1993) An overview: resistance to the Russian wheat aphid (Homoptera: Aphi didae) within the perennial Triticeae. J Econ Entomol 86:1609–1618

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

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

Masuelli RW, Tanimoto EY, Brown CR, Comai L (1995) Irregular meiosis in a somatic hybrid between *S. bulbocastanum* and *S. tuberosum* detected by species-specific PCR markers and cytological analysis. Theor Appl Genet 91:401–408

Moore G, Gale MD, Kurata N, Flavell RB (1993) Molecular analysis of small grain cereal genomes: current status and prospects. Bio/technology 11:584–589

Myburg AA, Cawood M, Wingfield BD, Botha A-M (1998) Development of RAPD and SCAR markers linked to the Russian wheat aphid resistance gene *Dn2* in wheat. Theor Appl Genet 96:1162–1169

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

Nkongolo KK, Quick JS, Limin AE, Fowler DB, Peairs FB, Myer WL (1990) Russian wheat aphid (*Diuraphis noxia*) resistance in wheat and related species. Can J Plant Sci 70:691–698

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

Penner GA (1996) RAPD analysis of plant genomes. In: Jauhar PP (ed) Methods of genome analysis in plants. CRC Press, Boca Raton, pp 251–268

Procunier JD, Knox RE, Bernier AM, Gray MA, Howes NK (1997) DNA markers linked to a *T10* loose smut resistance gene in wheat (*Triticum aestivum* L.). Genome 40:176–179

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

Stam P, Zeven A (1981) The theoretical proportion of the donor genome in near-isogenic lines of self-fertilizers bred by backcrossing. Euphytica 30:227–238

Venter E, Myburg AA, Botha A-M (1998) Characterisation of sequences linked to Russian wheat aphid resistance genes *Dn1*, *Dn2* and *Dn5*. In: Proc 9th Int Wheat Genet Symp, vol 3. pp 332–335

Weeden NF, Timmerman GM, Lu J (1994) Identifying and mapping genes of economic importance. Euphytica 73:191–198

Yu KF, Van Deynze A, Pauls KP (1993) Random amplified polymorphic DNA (RAPD) analysis. In: Griffin HG, Griffin AM (eds) Methods in plant molecular biology and biotechnology. CRC Press, Boca Raton, pp 287–301

Zemetra RS, Schotzko, DJ, Smith CM, Souza EJ (1990) Seedling resistance to the Russian wheat aphid in white wheat germplasm. Cer Res Commun 18:223–227