PROGRESS TOWARDS IDENTIFYING A MARKER FOR RUST RESISTANCE IN SUGARCANE VARIETY NCo376

JM BARNES[1] AND FC BOTHA[2]

[1]*South African Sugar Association Experiment Station, Private Bag X02, Mount Edgecombe, 4300*
[2]*Institute for Plant Biotechnology, University of Stellenbosch, Private Bag X01, Matieland, 7602*

Abstract

The observation was made recently at SASEX that a certain clonal derivative of NCo376, a variety typically resistant to rust (*Puccinia melanocephala*), shows a high incidence of infection with this pathogen. It is likely that a mutation of the original genotype occurred during propagation of NCo376. The assumption can therefore be made that any genetic differences between the two 'lines' have a high likelihood of being linked to rust resistance while the rest of the genetic background will consist of the normal NCo376 genotype. DNA samples from eight resistant and eight susceptible sources of material were screened using the RAPD assay, and this resulted in the identification of two different loci that proved to be reproducibly polymorphic between the two lines of NCo376. One of these has been cloned and the sequence determined, providing the opportunity for the fragment to be converted into a SCAR marker. Further characterisation of this fragment, and others that are subsequently identified, might allow development of a diagnostic marker and even elucidation of the mechanism of rust resistance (or individual factors governing resistance) in sugarcane.

Introduction

Near-isogenic lines (NILs) are a useful means of rapidly identifying DNA sequences that are linked to known plant genes. Pairs of NILs are developed by systematically back-crossing a line carrying a gene of interest (the donor parent) to a cultivated line having otherwise desirable characteristics (the recurrent parent). This process results in a line that carries a small segment of donor parent, including the target gene, in a genetic background that is almost exclusively that of the recurrent parent. Consequently, any genetic polymorphisms that are identified between the parent line and its NIL are likely to be linked to the target gene. NILs have been used successfully to identify markers for a variety of different genes (Martin *et al.*, 1991; Zhang *et al.*, 1997), as well as to study the nature of specific genes (Ignatius *et al.*, 1994).

Due to the complex nature of the sugarcane genome, the conventional development of NILs in this crop is highly impractical, if not impossible. However, the discovery of what appears to be a naturally occurring NIL may allow this approach of marker identification to be used in sugarcane after all. At SASEX, the variety NCo376 is used as a 'control' variety when assessing the performance of clones in the selection programme. NCo376 is typically resistant to rust (a disease caused by the fungal pathogen *Puccinia melanocephala*). However, in 1994 the observation was made that certain NCo376 clones growing at SASEX were particularly susceptible to this disease. A number of stalks from the typical NCo376 and the rust infected NCo376, respectively, were obtained from different locations on the Experiment Station, cut into setts, and planted in a single field. On reaching maturity, the propagated stools showed the same levels of infection as the plants from which they were taken. What was particularly distinctive about this phenomenon was that rust infected and rust free plants were growing in the immediate vicinity of each other.

The exact cause of the difference in phenotype is not known. However, it is likely that when a limited number of NCo376 stalks were used for bulking up the clones used for selection controls, one of the stalks had a mutation that resulted in the rust susceptible phenotype. This mutation would have been reproduced in each of the clones propagated from that stalk. Since these two 'lines' of NCo376 are most likely genetically identical with the exception of the mutation, they can essentially be considered a pair of NILs. This provides an ideal opportunity to investigate the genes and/or mechanisms underlying rust resistance in sugarcane. The random amplification of polymorphic DNA (RAPD) (Williams *et al.*, 1990) is a simple process based on the amplification of random stretches of genomic DNA. This technology is capable of revealing minor changes, as small as single base pair changes, in the genomes of related genotypes (Klein-Lankhorst *et al.*, 1991; Foolad *et al.*, 1993), making it an efficient means of gene tagging in NILs.

Methodology

Eight resistant and eight susceptible clones of NCo376 were selected to ensure that if any polymorphisms were identified, they were associated with resistance or susceptibility, and not individual-specific differences (Harvey *et al.*, 1994). DNA was extracted from the clones (Harvey and Huckett, 1998), and aliquots of DNA from the eight resistant and eight susceptible clones were then bulked to form two samples representative of the two phenotypes.

The DNA was subjected to RAPD amplification (Harvey *et al.*, 1994) using a range of random decamer primers (Operon Technologies), both individually and in pairs. Any primers yielding polymorphisms between the rust resistant and rust susceptible samples were then used to re-amplify the DNA to confirm the reproducibility of the polymorphisms.

One of the polymorphisms was cloned (PCR-Script SK (+) Cloning Kit, Stratagene) and sequenced (Perkin Elmer ABI Prism 310 Genetic Analyzer). The fragment was used in a Southern hybridisation (Hoisington, 1992) against the RAPD profiles of all the individual rust resistant and rust susceptible clones amplified with the same primers that were originally employed to generate the polymorphism.

Results and discussion

A total of 173 RAPD primers, or pairs of primers, were screened against the rust resistant and rust susceptible DNA bulks, and these amplified an average of 9,8 loci per reaction. Two of the loci amplified in the initial screening proved to be reproducibly polymorphic between the rust resistant and rust susceptible samples. The very low frequency of polymorphism between the two lines (0,1%) provides evidence that the susceptible line arose from a small mutation within the genome of NCo376. One of the polymorphisms, A4/17R, was selected for further characterisation. The name of this fragment was derived from the pair of primers used to amplify it (OPA4 and OPA17), with the 'R' indicating that it was amplified only from the clones with the rust susceptible phenotype.

To confirm that A4/17R was associated with rust susceptibility, the DNA from all 16 individual clones was amplified with the same primers, showing that the fragment was exclusively amplified in the susceptible clones (Figure 1A). When the gel shown in Figure 1A was blotted on a nylon membrane and probed with the fragment, strong hybridisation occurred to A4/17R as well as to a slightly larger fragment in the susceptible clones (Figure 1B). The larger fragment is likely to be a slightly longer version of A4/17R. These results clearly indicate the absence of the polymorphic fragment in the resistant clones.

Since the sequence of A4/17R has been determined, longer, more specific primers can be designed which will amplify the fragment as a SCAR (sequence characterised amplified region) (Paran & Michelmore, 1993). One of the advantages of a SCAR over a RAPD marker is that it will allow a more robust, reproducible assay for applications such as marker-assisted selection and mapping, should they prove applicable.

The sequence of A4/17R was compared against international databases of known genomic sequences but no significant homology was detected. However, the possibility does exist that this fragment is directly on a gene involved in rust resistance. The fragment can therefore be used to screen a genomic library in an attempt to isolate the gene.

The use of AFLPs (amplified fragment length polymorphisms) has the potential to identify additional polymorphisms between the resistant and susceptible clones of NCo376. Additional loci that are found to differ between the two lines can then be subjected to further characterisation, as with A4/17R. Information thus obtained can then determine the usefulness of the polymorphisms as markers for rust resistance in sugarcane.

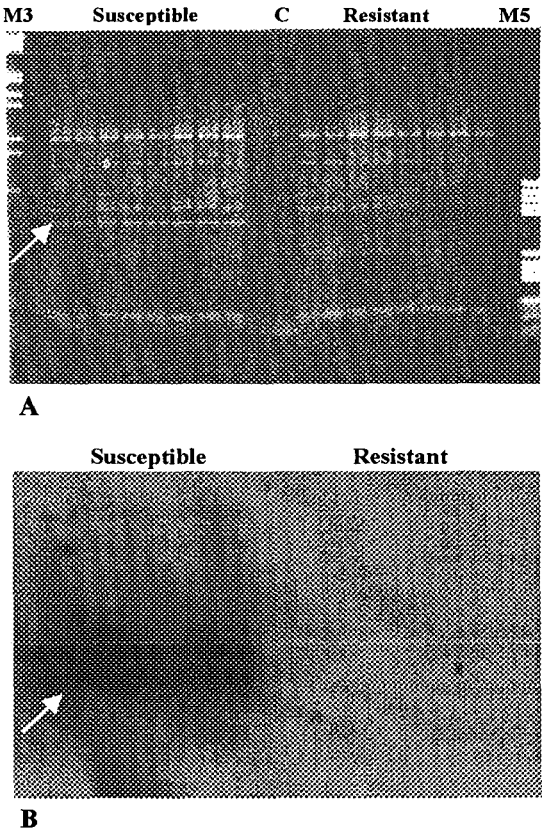

Figure 1. (A) RAPD profiles of the eight susceptible and eight resistant NCo376 clones, generated with primers OPA4 and OPA17. The arrow indicates the position of A4/17R, a 396 base pair fragment which was amplified in the susceptible clones only. Lanes marked M3 and M5 contain molecular weight markers, while lane C contains the PCR water control.
(B) Southern blot of the gel shown in (A) probed with fragment A4/17R. The probe hybridised strongly to itself (indicated with an arrow), as well as to a slightly larger fragment in the susceptible samples.

Acknowledgements

The authors wish to thank members of the Plant Pathology and Plant Breeding Departments at SASEX for planting the observation trials of rust resistant and rust susceptible NCo376. Appreciation is also extended to Dr Barbara Huckett of SASEX, for helpful comments and discussion.

REFERENCES

Foolad, MR, Jones, RA and Rodriguez, RL (1993). RAPD markers for constructing intraspecific tomato genetic maps. *Plant Cell Reports* 12: 293-297.

Harvey, M and Huckett, BI (1998). Preliminary genetic analysis of the sugarcane population AA40. *Proc S Afr Sug Technol Ass* 72: 315-316.

Harvey, M, Huckett, BI and Botha, FC (1994). Use of the polymerase chain reaction (PCR) and random amplification of polymorphic DNAs (RAPDs) for the determination of genetic distances between 21 sugarcane varieties. *Proc S Afr Sug Technol Ass* 68: 36-40.

Hoisington, D (1992). *Laboratory Protocols*. CIMMYT Applied Molecular Genetics Laboratory, Mexico.

Ignatius, SMJ, Chopra, RK and Muthukrishnan, S (1994). Effects of fungal infection and wounding on the expression of chitinases and β-1,3 glucanases in near-isogenic lines of barley. *Physiologia Plantarum* 90: 584-592.

Klein-Lankhorst, RM, Vermunt, A, Weide, R, Liharska, T and Zabel, P (1991). Isolation of molecular markers for tomato (*L. esculentum*) using random amplified polymorphic DNA (RAPD). *Theor Appl Genet* 83: 108-114.

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

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

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

Zhang, G, Bharaj, TS, Lu, Y, Virmani, SS and Huang, N (1997). Mapping of the *Rf-3* nuclear fertility-restoring gene for WA cytoplasmic male sterility in rice using RAPD and RFLP markers. *Theor Appl Genet* 94: 27-33.