Marker Assisted Selection of Sunflower Rust Resistance Genes

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Abstract

The production of sunflower lines with durable resistance to rust infection is dependent on an efficient and reliable method for the identification of individual rust resistance genes. PCR based markers linked to the R1 rust resistance gene and a rust resistance gene derived from the 'Advance' hybrid have been developed and analysis of these markers in a range of sunflower germplasm indicates a close relationship between the presence of the PCR marker(s) and the predicted presence of the resistance gene. Utilisation of these markers in a sunflower improvement program is anticipated to facilitate the development of durable resistance to rust in sunflower germplasm by successfully verifying the presence of these genes.

Introduction

Rust, caused by the fungal pathogen *Puccinia helianthi* Schw., can cause major yield losses to sunflower producers. Hybrid cultivars which contain simple or complex genetic resistances where resistance genes are added sequentially over time tend to break down to new races of rust within 5 years of release. Pyramiding of rust resistance genes into a single genetic background is anticipated to be the most effective strategy for producing sunflower germplasm with durable resistance to rust.

Molecular biology offers a number of tools that will facilitate breeding for disease resistance. Molecular markers have certain advantages over the use of phenotypic screening procedures such as the ability of screening seedlings in the absence of the disease, at times of the year when the pathogen is absent, and especially for following introgression of genes into genotypes already resistant to the available pathotypes. This last factor is important for our present strategy for controlling sunflower rust, that is, the creation of genotypes where as many of the known rust resistance genes are combined or pyramided.

Molecular markers for two genes conferring resistance to sunflower rust are reported in this paper. This is the first report for molecular markers for rust resistance genes in sunflower and follows recent reports of the identification of markers for genes conferring resistance to sunflower downy mildew (Mouzeyar *et al.*, 1995). The R1 gene was chosen for marking because it is present in a large number of sunflower lines which could therefore be used to test the robustness of the marker. The gene conferring resistance in the sunflower hybrid "Advance" was chosen because this gene confers resistance to most of the pathotypes of sunflower rust in Australia.

Materials and Methods

RAPD markers have been previously identified (Lawson *et al.*, 1996 and Lawson *et al.*, manuscript in preparation) to the R1 rust resistance gene, resistant to Australian rust race 0, North American rust race 1 (Kochman and Goulter, 1985) and the gene derived from the commercial hybrid 'Advance' (Pioneer Overseas Corporation), resistant to all present races of rust in Australia except the rust race 'Advance' (*unpublished data*).

Conversion of three of the RAPD markers into the PCR based markers SCARs, as described by Paran and Michelmore (1993) involved cloning of the respective RAPD marker and partial sequencing of the marker to allow the design of specific PCR primers.

Under specific PCR conditions, each marker band was amplified in genomic DNA from a range of sunflower germplasm available in Australia. Presence or absence of the marker band was verified by running the amplification reactions on agarose gel electrophoresis and visualising over UV illumination after staining with ethidium bromide.

Results

Using the PCR conditions described for SCAR analysis, primer pairs for the amplification of the marker associated with the R1 gene, and primer pairs for marker 1 associated with the gene from 'Advance' , amplified a single band of 950bp and 600bp (respectively) in their respective resistant parents. For the SCAR primer pair for marker 2 associated with the resistance gene from 'Advance' alleles were amplified in both parents as size variants of 950bp in the resistant parent and 850bp in the susceptible parent. Therefore, in two cases, the resistance loci can be scored with dominant markers while in the other case the SCAR is co-dominant. In all cases the SCAR marker band in the resistant parents is the same size as the original RAPD marker.

To assess the potential usefulness of the SCAR markers for detecting the respective resistance gene, amplification of the specific marker band was tested in a wide range of sunflower germplasm. The lines include breeding lines, differential lines and current susceptible and resistant hybrids (Table 1).

For each of the lines tested using the SCAR primers for the R1 resistance gene and the gene derived from the 'Advance' hybrid, the presence of the marker band corresponded reliably with the predicted presence of the gene in that germplasm. The band did not amplify in germplasm not thought to contain the gene. However, in other cases the marker bands did amplify in material in which it is uncertain whether the gene is present or not. This is probably due to the presence of other genes for rust resistance in these lines masking the presence of the targeted rust resistance gene.

Discussion

The utility of the SCAR markers reported here has been verified by screening DNA extracted from a number of sunflower lines considered by pedigree or reaction to pathotypes of sunflower rust to have or lack the respective genes. The R1 SCAR amplified in lines thought to possess the R1 gene such as S37-388RR, MC69-17-8-1-1, CM90RR and F164A as well as the 953-accessions which are considered to be original sources of this gene. The SCAR did not amplify in lines such as MC29-3-1-3-2-1 (R2 gene source) and PhRR3 (R3 gene source). Of special interest was the differential presence of the marker in the inbred lines RHA 278 and RHA 279. These lines source to a single F5 progeny where plants resistant to rust were bulked to create RHA 279 while RHA 278 consisted of plants resistant to downy mildew (U.S.D.A. Release Notice, 1975). These two lines have been shown to be very similar genetically (Korell *et al.*, 1992) hence the polymorphism with regard to the R1 SCAR suggest that the marker is in close linkage to the R1 gene.

The SCARs for the gene from the hybrid 'Advance' are flanking the locus. The interval between them of approximately 20cM means that selection with >90% probability of recovering the gene must be made on the presence of both markers (Tanksley, 1983). These SCARs did not amplify genomic DNA extracted from a diverse range of sunflower lines including the sunflower rust differentials but did amplify genomic DNA from a number of genotypes provided by Pioneer Seeds (Pioneer Overseas Corporation). The presence or absence of the gene derived from 'Advance' was verified in the genotypes from rust reaction scores. Failure of the SCARs to amplify genomic DNA from the differential lines supports the contention that the gene in 'Advance' was not in common use in Australia before release of this hybrid. It also explains why 'Advance' was resistant to all the pathotypes of sunflower rust recognised in Australia at the time of the hybrids release. Assuming that the sunflower:*Puccinia helianthi* pathosystem conforms to a gene-for-gene model where dominant resistance genes in the host confer

resistance to pathotypes possessing the corresponding avirulence genes then a sunflower hybrid (eg 'Advance') possessing a single resistance gene can be resistant to a large number of pathotypes if each contains the corresponding avirulence gene. This resistance gene will therefore be very useful in our strategy of developing durable rust resistance through controlled recombination of genes since the corresponding avirulence gene is common in the pathogen population in Australia.

Molecular markers can also help to infer the resistance genotype of lines of unknown pedigree. For example, Miller *et al.* (1988) demonstrated the presence of the R4 gene in HA-R1, HA-R3, HA-R4 and HA-R5 by screening respective crosses with N.A. Race 4 of sunflower rust. The ability of these sunflower lines to further differentiate pathotypes within the sunflower population suggested that these lines contained other genes for resistance to sunflower rust as well. The presence of the R1 SCAR in HA-R1 suggests that this line also possesses the R1 gene. Presence of this gene would not have been detected in crosses made by Miller et al. (1988) because it does not confer resistance to N.A. Race 4. Of course, presence of the R1 gene can only be confirmed by making and screening the relevant crosses.

We believe that the use of RAPDs and bulk segregant analysis in the development of trait specific markers such as SCARs provide an efficient method of marker development. The utility of these markers will be further enhanced by integration onto a genetic map. This will provide evidence that the genes are part of multi-allelic loci or exist in tight linkage groups. Both of these phenomena have important implications on our ability to pyramid genes.

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Table 1: Correlation of the presence of the three SCAR markers with the presence of either the R1 or the 'Advance' genes in a number of sunflower genotypes.

	R1 Gene		'Advance' Gene		
Sunflower Lines	Presence of Gene	Presence of SCAR band	Presence of Gene	Presence of 'A' SCAR 1	Presence of 'A' SCAR 2
Rust Differentials:					
S37-388	-	-	-	-	-
S37-388RR	+	+	-	-	-
MC69-17-8-1-1	+	+	-	-	-
F164A	+	+			
CM90RR	+	+			
MC29-3-1-3-2-1	-	-	-	-	-
953-88-3-1-54	+	+			
953-102-1-1-41	+	+	-	-	-
PhRR3	-	-	-	-	-
HA-R1	?	+	-	-	-
HA-R2	?	-	-	-	-
HA-R3	?	-	-	-	-
HA-R4	?	-	-	-	-
HA-R5	?	-	-	-	-
Inbreds:					
RHA 278	-	-			
RHA 279 RP#	+	+			
HA 89	-	-	-	-	-
'Advance' RP#			+	+	+
'Advance' SP			-	-	-
Hybrid Cultivars:					
Hysun 32	+	+			
Hysun 33	+	+	-	-	
Hysun 45CQ	?	-			
Suncross 40R	?	+			
'Advance'	?	-	+	+	+
Pioneer Genotypes					
GEN1			+	+	+
GEN2			+	+	-
GEN3			-	-	-
GEN4			-	-	+
GEN5			+	+	+
GEN6			+	+	+
GEN7			+	+	+
GEN8			+	+	+

Resistant Parents, sources of SCAR markers

RP (resistant parent), SP (susceptible parent), + (gene or marker band present), - (gene or marker band absent), ? (presence of gene uncertain).