A MOBILE GENETIC UNIT ASSOCIATED WITH DISEASE RESISTANCE IN SUNFLOWER.

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Summary:

The discovery of transposable elements in many eukaryotic organisms, including plants, and the role these elements may play in gene regulation and evolution has generated a lot of interest and research. Computer-aided analysis of the sequence of the SCAR marker $SCX20_{660}$, linked to the sunflower rust resistance gene R_{Adv}, identified significant similarities between the marker sequence and two conserved domain motifs of retrotransposons. Analysis of the nucleic acid binding protein motif indicates that this motif is present in the sunflower genome in low copy number. Association of the motif with resistance to Pathotype Aus15 of the sunflower fungal pathogen *Puccinia helianthi* is assessed.

Introduction:

Barbara McClintock first described a mobile genetic unit while studying the inheritance of kernal mottling in maize. McClintock observed that a genetic element associated with chromosome breakage did not have a constant location, and occassionally "jumped" to a new position. This phenomenon became known as transposition and the element responsible was called a transposable element or transposon. Since her groundbreaking work on transposons, it has been suggested that these units are an integral part of eukaryotic genomes and may play an important role in gene regulation and evolution.

Transposons can insert themselves at different locations within the genome and may affect the functions of genes with which they become associated. A number of different classes of mobile elements have been discovered, those that transpose via a DNA-DNA mechanism (transposon) and those that form new copies of themselves via an RNA intermediate using reverse transcriptase (retrotransposon). A number of different classes of retrotransposon have been identified. These are determined according to the organisation of the domains that encode for the *gag* protease, integrase, reverse transcriptase, RNase H and *env* proteins that are necessary for reverse transcription and integration of each unit into the host genome (for reviews see Kumar, 1998 and Wessler 1998).

Both transposons and retrotransposons have been discovered in insects, yeasts, nematodes, filamentous fungi, plants and mammals. They have been found to be diverse and ubiquitous in plant genomes. The copy number of retrotransposon elements or retroelements, is quite variable across different genomes with insects containing as few as 10-80 copies. In contrast, the *tnt1* element of tobacco (Grandbastien *et al.,* 1989) and the *wis-2* element of wheat (Harberd *et al.,* 1987; Moore *et al.,* 1991) are present as several hundred copies, while the copy number of the *del1* element from lily can be as high as 13,000 and even higher in other species of *Lilium* (Joseph *et al.,* 1990).

Most plant retroelements are transcriptionally inactive. Those that are active, are believed to be activated in response to stress, such as that encountered during pathogen attack, or cell/tissue culture. It has been postulated that this reaction of the host genome to outside stimuli may aid in survival by regulating gene expression (leading to gene evolution) and creating genetic diversity. This ensures an increase in adaptability in extreme environments. More than 100 ‘normal’ plant genes are reported to have mobile elements or remnants of elements located close to their coding regions.

This paper reports the discovery of a marker sequence, linked to a sunflower rust resistance gene, which has retrotransposon-like coding domains. We report the domains identified and copy number of the element within a range of sunflower genotypes.

Materials and Methods:

SCAR-PCR Identification of the SCAR marker $SCX20_{660}$ completely linked to the R_{Adv} gene was described by Lawson *et al.,* (1998). The rust resistance gene R_{Adv} in sunflower confers resistance to all Australian pathotypes except for Aus15, 21 and 23 (Kong *et al.,* 1999).

Southern hybridisation Analysis of transposon copy number in the sunflower genome was performed by southern hybridisation following standard procedures. Typically 12 μg of total genomic DNA (genotypes CM303, *cms*HA89, B89, P1, P2, HA-R2, HA-R4 and HA52) was restriction digested with the enzymes *Hind* III, *Eco* RI, *Eco* RV, *Dra* I, *Hae* III and *Bam* HI. The resulting fragments were transferred to Hybond N+ nylon membrane using 20X SSC and hybridised to the nucleic aciding binding protein $CX_2CX_4HX_5C$ motif sequence amplified from the marker sequence using the following primers:
GAG-F 5' TTGTTGTTTGCTGTTGTAGCTAGT 3'
GAG-R 5' TAATGAAGAAGAGTGTAGAAACAAAG 3'.
The probe was radioactively labelled using the Megaprime™ DNA labelling system (Amersham LIFE SCIENCE, England).

Sequence analysis Deduced amino acid sequences and the database sequence comparisons using the BLASTX function (Altschul *et al.,* 1997) were performed using the GCG program available on WEBANGIS at the University of Sydney. Multiple sequence alignments were compiled using the EclustalW and PrettyBox functions on WEBANGIS. Accession numbers for the database query sequences are Arabidopsis (AF128395), Rice (AF111709) and Sorghum (AF061282).

Results:

The SCAR marker $SCX20_{660}$, was found to perfectly co-segregate with the sunflower host rust resistance gene R_{Adv} in 270 F2 individuals (Lawson *et al.,* 1998 and unpublished data).

Comparison of the 660bp R_{Adv} marker sequence with known sequences on the Genbank database identified significant amino acid similarity (approximately 30%) between the marker sequence and conserved motif sequences commonly represented in retrotransposons (Figure 1A).

Translation of the 660bp sequence identified a single uninterrupted open reading frame encoding 188 amino acids which contained the retrotransposon motifs, '$CX_2CX_4HX_5C$', a nucleic acid binding domain similar to that encoded by the *gag* region of retroviruses, and 'VLFDSGA', a putative protease active site (P). The predicted structure of the R_{Adv} retroelement is represented in Figure 1B. Due to the short length of the sequence obtained so far, it is not possible to identify any other motifs including the primer binding site or the LTR regions.

Plant retrotransposons have been shown to be present in some genomes in large copy numbers. Southern hybridisation analysis using the nucleic acid binding protein motif sequence as a probe was undertaken to assess the copy number of this unit in the sunflower genome. Across a range of lines, the probe was found to hybridise to between 4 and 17 fragments indicating that this is a relatively small copy number unit in the sunflower genome. Although no polymorphic fragments could be found between rust resistant and susceptible lines, the probe was also tested in 70 individuals of the F2 population tested for the original SCAR marker. The probe hybridised

intensely to three fragments but in no case was there a polymorphism associated with resistance (data not shown). This was also the case when the full length marker (ie the 660bp sequence) was used as a probe. Across five restriction enzymes, no polymorphism between the resistant and susceptible parents for the F2 cross was observed. This would indicate that the polymorphism detected using the SCAR marker could be due to a polymorphism within the primer binding site for the PCR rather than a deletion or addition in the genome in that genomic area. The specificity of the SCAR marker to the R_{Adv} gene in sunflower material is supported by the lack of PCR priming in lines that do not contain the R_{Adv} gene (Lawson *et al.,* 1998).

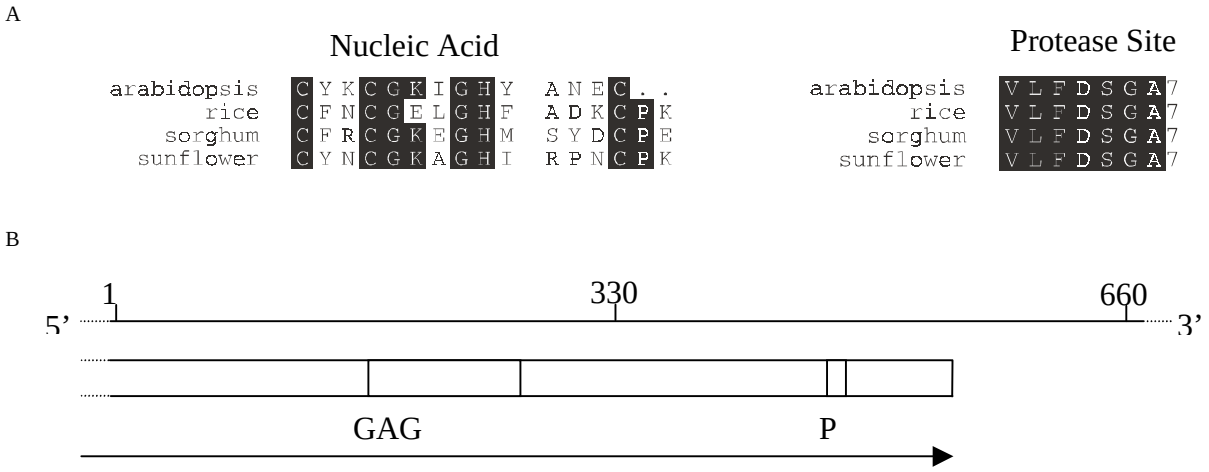

Figure 1:

A: Multiple sequence alignment of the gag polyprotein motif and the putative protease active site motif and the sunflower sequence reported in this paper.

B: Predicted structural organisation of the retrotransposon-like element associated with the R_{Adv} gene. The diagram indicates the *gag* polyprotein region and the putative protease active site (P). The arrow indicates he long open reading frame.

Discussion:

This is the first report of a retrotransposon-like sequence identified in the sunflower genome. A computer-aided search of Genbank has revealed that the R_{Adv} gene marker sequence has similarities with two conserved domains present in retrotransposon-like elements. Elements corresponding to the nucleic acid binding protein of the *gag* polyprotein region and the putative protease site are thought to be regulatory sequences for mRNA transcription. Further sequence analysis needs to be undertaken to determine which class of retrotransposon this element belongs to. The identification of a transposable element within sunflower is not surprising. It has been shown that many repetitive intergenic genomic sequence belongs to transposons or remnants of transposable elements. SanMiguel *et al.,* (1996) reported that maize contains approximately 60-80% repetitive DNA and that retrotransposable element sequences account for approximately 50% of the nuclear DNA.

The cultivated sunflower genome (*Helianthus annuus* L.) has approximately 12 pg DNA per haploid genome (Arumuganathan and Earle, 1991). It is anticipated that a high percentage of it will consist of repetitive DNA, as most complex plant genomes are approximately 70% repetitive sequence (Flavell et al., 1977). However, the low copy number of the nucleic acid binding protein motif within the sunflower genotypes tested suggests this class of element is not frequent within the genome and is unlikely to be transcriptionally active. The presence of this unit in a number of genetically diverse lines of sunflower indicate that the unit has not been recently inserted into the genome.

The presence of transposable elements associated with genes for disease resistance have been reported elsewhere. The Tnt1 element of tobacco (Grandbastien *et al.,* 1997) was shown to be activated in response to pathogen defense, while Bhattacharyya *et al.,* (1997) found the Tgm*r* transposon associated with the *Rps1-k* allele in soybean. The inability to detect linkage between this element and disease resistance in sunflower by southern hybridisation analysis is unlikely to be due to the lack of an association between the marker and the trait. An association can be shown in the fact that the SCAR marker completely cosegregates with the resistance trait in an F2 population of 270 individuals (unpublished data). The inability to detect polymorphism in the Southern may be due to the use of the nucleic acid protein motif sequence (ie $CX_2CX_4HX_5C$) as a probe rather than a sequence based on the conserved motif of the reverse transcriptase. It has been suggested that sequence divergence in the reverse transcriptase sequence of these elements can contribute to the evolution of new sequences and perhaps new genes. Predicted amino acid levels of a group of reverse transcriptase genes from retrotransposons of potato were found to have similarities between 5-75% (Flavell *et al.,* 1992). Therefore, this seems to be the highly variable region of the element and the most likely to detect polymorphisms between closely related sequences. The marker closely linked to the *Rps1-k* allele of soybean was located within the reverse transcriptase domain of the retrotransposon (Bhattacharyya *et al.,* 1997).

Determining the class of transposable element for the R_{Adv} marker, and establishing its association with disease resistance will require further analysis. Nonetheless, this marker has been shown to have similarity with a retrotransposable-like element, and is known to be located within a cluster of rust resistance genes (Lawson *et al.,* 1999). Further evaluation of this element is anticipated to assist in characterisation of sunflower rust resistance genes.

Acknowledgements:

The authors wish to acknowledge the financial support received from the Cooperative Research Centre for Tropical Plant Pathology (now Tropical Plant Protection) and the Australian Grains Research and Development Corporation.

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