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SINGLE NUCLEOTIDE POLYMORPHISMS IN BARLEY USING TETRA-ARMS PCR

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Single Nucleotide Polymorphisms (SNPs) in Barley: the Use of Tetra-Primer ARMS-PCR

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1. INTRODUCTION

Single nucleotide polymorphisms (SNPs) are the most abundant form of DNA polymorphism. In various studies in plants, there appears to be one SNP roughly every 100bp. This abundance allows the construction of high-density genetic maps offering great potential to detect associations between allelic forms of a gene and phenotype. Thus SNPs can be used as simple genetic markers for many breeding applications, for population studies and for fingerprinting. In addition, the dramatic increase in the number of DNA sequences submitted to the databases has made it possible to identify many SNPs for several crops without the need for sequencing (a process known as database mining or SNP e-mining). The availability of expressed sequenced tag (EST) databases makes it possible to target the polymorphisms to functional regions of the genomes and even to specific genes. Many methods have been developed for SNP genotyping. Many of these detect single nucleotide variations which generally requires expensive DNA sequencing equipment to perform single base extension, or pyrosequencing, of the PCR products. Others are based on hybridisation assays that require radioactivity or reporter molecules, such as the "TaqMan" system. These methods are developed for high throughput analysis, with cost being a secondary issue. The expense and practicality of such technologies have so far limited the uptake of this class of DNA markers. A simple and economical method involving a single PCR followed by separation on agarose gels is reported here for SNPs genotyping of barley. Using the tetra-primers ARMS-PCR procedure, we have been able to assay unambiguously five SNPs in a set of 132 varieties of cultivated barley. The results show the reliability, low cost and ease of use of this technique.

2. METHODS

SNP Information and Plant Material

Information and sequences of nine barley loci (MWG2062, ABC465, MWG2218, ABG601, MWG502, ABG704, MWG2029, ABC156 and MWG801) were kindly supplied by Montana State University; http://hordeum.oscs.montana.edu/locus/index.html). DNA extracts of the five barley varieties Baronesse, Karl, Lewis, Morex and Steptoe (previously surveyed for SNPs discovery, Kanazin *et al.* 2002, Plant Mol. Biol. 48, 529) were also provided. In addition, DNA samples extracted from bulked seeds from a further 132 spring and winter barley varieties were tested.

Tetra-Primer ARMS-PCR Procedure

The tetra ARMS PCR procedure (Ye *et al.* 2001, Nucleic Acids Research 29, e88) employs four primers to amplify a larger fragment from DNA that contains the SNP and amplicons representing each of the two allelic forms (Figure 1). Primers can be designed to amplify fragments of differing sizes for each allele band that can be easily resolved using agarose gel electrophoresis. To increase the specificity of the reaction, a mismatch is introduced at the 3' end of each of the two allele-specific primers.

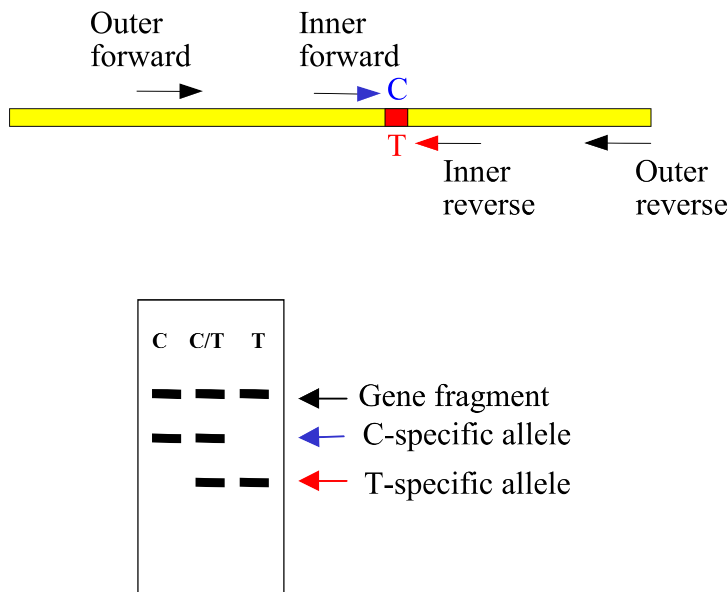

Figure 1. Diagrammatic representation of tetra ARMS PCR procedure

Primers were designed using the primer design programme made available by Ye et al. (2001) (http://cedar.genetics.soton.ac.uk/public_html/primer1.html). The primers were designed by limiting the fragment sizes to the range of 150-400 bp and the ratio of the allelic bands to 1.2-1.5. Default settings were used for other parameters. PCR was carried out following the protocols of Ye et al. (2001) with slight modifications and the PCR products were electrophoresed in a 1.5 % agarose gel and stained with ethidium bromide.

To validate the accuracy of the tetra-primer ARMS-PCR method, primer sets were tested on the five barley varieties originally utilised for SNP discovery by Kanazin *et al.* (2002) (see above) by direct sequencing of PCR products from each variety. Each locus was amplified using primer sequences published in GrainGenes (http://wheat.pw.usda.gov) and the fragments were sequenced directly. The types of polymorphisms found included transitions and transversions, as well as insertion-deletion events. The nine loci given above were chosen from the 54 available because they permitted the design of suitable primer sets for tetra-primer ARMS-PCR SNP detection in agarose gels. Only polymorphisms within a 250-500 bp size range were considered. Although most of the loci contained multiple polymorphisms, we examined only one SNP per locus. As the allelic composition of the five test varieties at each locus was known, it was possible to validate the tetra ARMS PCR primers by first analysing these five varieties. A set of primers was considered as being validated when both allelic bands and the outer fragment, all of the expected sizes, were visualised. The optimised protocol was then used to genotype the 132 varieties.

3. RESULTS

Only five of the nine loci could be successfully analysed on all 132 varieties using agarose gels to separate and visualise the PCR products. This was though to be due to primer design difficulties, the quality of the sequence analysed and/or the size of the product. The results of the SNP analysis are summarised in table 1 and will be discussed more fully in the presentation.

Table 2. Summary of the SNP genotyping of 132 barley varieties

Locus	Chromosome	Polymorphism[a]	Ratio of allelic compositions [b]
MWG2062	7H	R	80 G : 50 A : 2 H
ABC465	7H	Y	123 T : 9 C
MWG2218	6H	S	82 C: 50 G
MWG502	5H	R	78 A :54 G
ABG601	4H	Y	89 C : 40 T : 3 H

[a] R =A,G; Y =C,T; S =G,C
[b] H = heterozygote

4. DISCUSSION

Tetra-primer ARMS PCR is a rapid, simple, robust, low cost and easy to use method for SNP genotyping, which could be used in most laboratories. In this work we have designed the primers in order to amplify fragments that differ in size sufficiently to be easily resolved by agarose gel electrophoresis. This modification from the original method makes the technique more readily usable across a range of research facilities.

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