Marker-assisted selection of genetic variants for seed trypsin inhibitor content in peas

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Summary

Despite their low contents in seeds, protease inhibitors in pea *(Pisum* sativum L.) have been shown to be important in negatively affecting the performance of pig or poultry. The quantification of these inhibitors by chemical methods is time-consuming and expensive, prompting the development of simple markers to boost progress in breeding programs. Gene polymorphism linked to variation in seed trypsin inhibitor activity has allowed the definition of primers for polymerase chain reaction (PCR) assays, which can be exploited in breeding programs and offer an efficient and cheap pre-screening procedure.

Introduction

The development of simple markers is very desirable to boost progress in breeding. The easier the screening, the more diverse and numerous the crosses that can be manipulated by breeders. Several legume seed constituents, that have been demonstrated to possess antinutritional properties for pig or poultry (3, 4), represent targets for the development of facile markers. The reduction of tannins in pea and faba bean seeds by breeding is simplified by the fact that the genes involved are recessive genes with a clear-cut pleiotropic effect on flower color. However, the situation is more complex for the removal or reduction of other anti-nutritional components, such as trypsin inhibitors (TI) in pea. Even though simplified chemical methods have been developed for this class of seed proteins (5), these methods still prove expensive for the screening of numerous genotypes. Expensive thermal treatments can inactivate seed inhibitors, but it has been shown that natural genetic variability may be exploited for improved animal performance (4, 7). Such genetic variability motivated a search for facile markers to assist breeding.

Materials and methods

The development of a set of diagnostic PCR primers, based on sequence polymorphism in promoter and coding regions of pea TI genes from a number of pea genotypes, has been described (6). Recombinant inbred lines (RILs) were derived from a cross between cv. Terese [low trypsin inhibitor activity (TIA)] and cv. Champagne (high TIA). A total of 168 F_7 RILs were screened, using this set of PCR primers and, in addition, using a rapid biochemical method (5) to determine TIA value. The sequences of the primers At12, At13, At14, At5 and At8rev and the conditions for their combined use as a cocktail for PCR assays have been reported (6).

Results and Discussion

The genetic basis for TIA variation in pea seeds, and the control of quantitative variation for this trait by a main quantitative trait locus (QTL) closely linked to TI genes at the *Tri* locus, has been described (1). These data identified 'candidate TI genes', whose variability within *Pisum sativum* L. could explain seed TIA. The structures of TI coding sequences were described first in one genotype (2), and this knowledge was extended recently to promoter and coding regions of these genes in additional genotypes; in particular, inter-

genotypic sequence polymorphism was defined (6). PCR primers were designed to distinguish gene polymorphisms between pea lines having high or low TIA, and various PCR patterns were described for a number of lines (6). These patterns were scored first on 95 lines from a collection of pea genetic resources, where a correlation between a 646 bp PCR product and low TIA values was observed (6). We now examine the utility of these markers for breeding programs and report the results of testing this set of PCR primers on a population of 168 RILs from a cross between two pea lines having high or low TIA.

When the population of RILs derived from parents having high or low TIA was screened for PCR product size and TIA, in no case did the 646 bp band result from a line with a high TIA (Figure 1). PCR markers based on the *Tri* locus are thus linked to TIA in pea seeds, providing strong markers directly located to TI genes and based on a simple PCR reaction. This type of marker promises to be of enormous value to breeders, especially if the DNA extracted from populations can be screened for additional markers simultaneously.

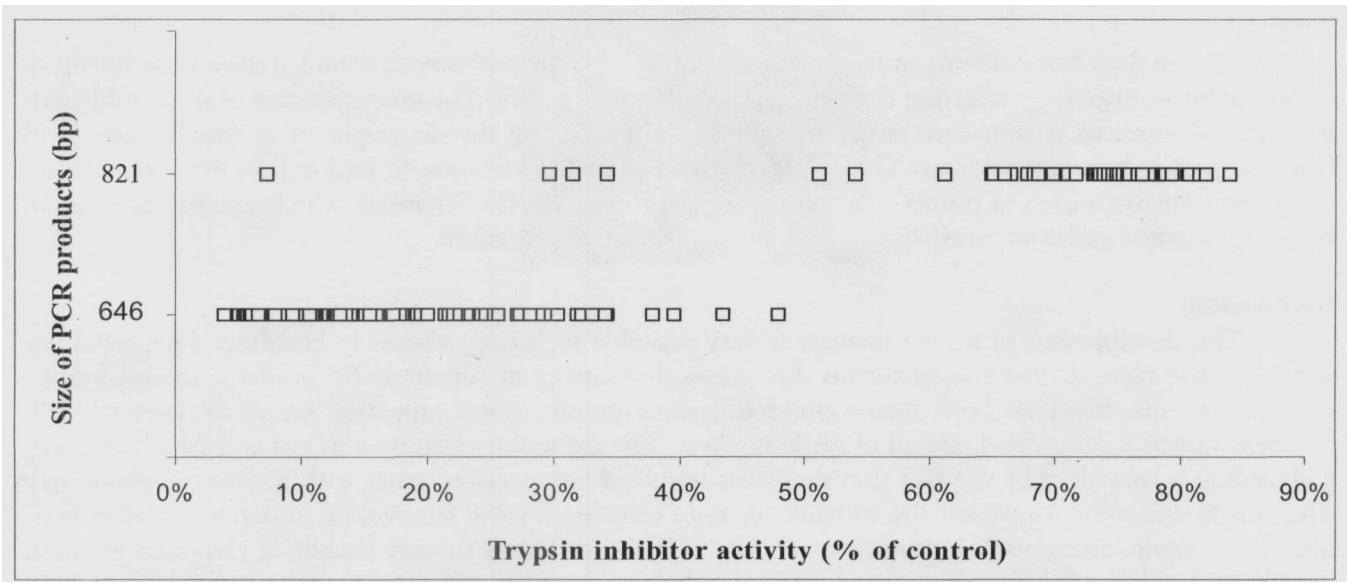

Fig. 1. Summary of PCR results for a population of 168 recombinant inbred lines from the cross Terese (low TIA) X Champagne (high TIA). Scores for two PCR bands of 646 and 821 bp are shown (ordinate). The TIA of each line is indicated on the abscissa; the higher the percentage, the higher the corresponding TIA.

The strategy of marker-assisted breeding promises to be valuable because of potential saving in the cost of phenotyping/genotyping. In some cases, it can also reduce errors due to environmental effects on gene expression. We illustrate here how knowledge of genes can be used to provide simplified tools to breeders that can be used to facilitate selection of variants for legume seed composition. The present rapid development of finer genetic maps, with new types of markers, as well as the development of robotics for genomics, should make molecular approaches for breeding even more efficient in the future. Selection may then be based on markers that are very close to the character and, in addition, numerous seed compounds may be screened simultaneously.

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