COMPARATIVE GENOME ANALYSIS AND RESISTANCE GENE MAPPING IN GRAIN LEGUMES

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Abstract

Using DNA markers and genome organization, several important disease resistance genes have been analyzed in mungbean (*Vigna radiata*), cowpea (*Vigna unguiculata*), common bean (*Phaseolus vulgaris*), and soybean (*Glycine max*). In the process, medium-density linkage maps consisting of restriction fragment length polymorphism (RFLP) markers were constructed for both mungbean and cowpea. Comparisons between these maps, as well as the maps of soybean and common bean, indicate that there is significant conservation of DNA marker order, though the conserved blocks in soybean are much shorter than in the others. DNA mapping results also indicate that a gene for seed weight may be conserved between mungbean and cowpea. Using the linkage maps, genes that control bruchid (genus *Callosobruchus*) and powdery mildew (*Erysiphe polygoni*) resistance in mungbean, aphid resistance in cowpea (*Aphis craccivora*), and cyst nematode (*Heterodera glycines*) resistance in soybean have all been mapped and characterized. For some of these traits resistance was found to be oligogenic and DNA mapping uncovered multiple genes involved in the phenotype.

1. INTRODUCTION

DNA marker technology has profoundly transformed plant genetics and breeding. Using DNA markers, highly saturated linkage maps of important crop species can be quickly constructed and used to characterize genome organization, locate genes of interest, and carry out marker-assisted selection for traits of economic importance. Two areas that have been especially fruitful are comparisons between the genomes of related, but sexually incompatible taxa, and the mapping of genes that underlie disease resistance phenotypes. Significantly, DNA markers can be used to identify and characterize genes involved in both monogenic and polygenic traits. This has been especially useful in the analysis of quantitative disease resistance characters in plants.

2. RESULTS AND DISCUSSION

2.1. Construction of DNA marker maps for mungbean and cowpea

A genetic linkage map of mungbean consisting of 172 markers including 151 random genomic DNA and 20 cDNA RFLP loci and one pest (*Callosobruchus*) resistance locus was constructed [1]. All but six markers were assigned to 11 coherent linkage groups, plus three small groups with four markers or less. The linked loci covered 1570 centiMorgans (cM) with an average distance of 9 cM. A majority of the mapped loci (121 out of 171) corresponded to single- and low-copy sequences as previously defined. Markers detecting more than one locus, exhibiting aberrant ratios and dominant/null phenotypes were detected as well in the current map. Although the number of linkage groups in the present map does not coincide yet with the 11 known chromosomes of mungbean, the overall appearance of the 11 coherent linkage groups in the map corresponds closely to the karyotype description reported previously.

A genetic linkage map for cowpea was constructed using 58 plants from an F2 population derived from a cross between an elite cowpea line (IT2246-2) and a non-cultivated wild relative (TVn 1963) [2]. These two lines share the same primary gene pool, although the

F1 hybrid between them showed partial pollen fertility. Between these two genotypes, restriction fragment length polymorphisms (RFLPs) were detected by about 22% of the genomic clones from various sources. Seed coat texture and pod shattering were also scored on the F2 plants. Eighty three loci (comprising 79 genomic, 5 RAPDs, 4 cDNA and 1 morphological trait) were distributed on 10 linkage groups which span 680 cM.

2.2. Comparative genome analysis between mungbean and cowpea

Genome relationships between mungbean and cowpea based on the linkage arrangement of RFLP markers were investigated [3]. A common set of probes derived from cowpea, common bean (*P. vulgaris*), mungbean, and soybean (*G. max*) *Pst* I genomic libraries were used to construct the genetic linkage maps. In both species, a single F2 population from a cross between an improved cultivar and a putative wild progenitor species was used to follow the segregation of the RFLP markers. Approximately 90% of the probes hybridized to both mungbean and cowpea DNA, indicating a high degree of similarity in the nucleotide sequences among these species. A higher level of polymorphism was detected in the mungbean population compared to the cowpea population. Loci exhibiting duplications, null phenotypes and distorted segregation ratios were detected in both populations. The mungbean and cowpea genomes were compared based on the copy number and linkage arrangement of 53 markers mapped in common between the two species. Results indicate that nucleotide sequences are conserved, but variation in copy number were detected and some rearrangements in linkage order appeared to have occurred since the divergence of the two species. Entire linkage groups were not conserved but several large linkage blocks were maintained in both genomes.

2.3. Comparative genome mapping among grain legumes

A set of 219 DNA clones derived from mungbean, cowpea, common bean, and soybean was used to generate comparative linkage maps among mungbean, common bean, and soybean [4]. The maps allowed an assessment of linkage conservation and colinearity among the three genomes. Mungbean and common bean, both of the subtribe Phaseolinae, exhibited a high degree of linkage conservation and preservation of marker order. Most linkage groups of mungbean consisted of only one or two linkage blocks from common bean (and vice versa). The situation was significantly different with soybean, a member of the subtribe, Glycininae. Mungbean and common bean linkage groups were generally mosaics of short soybean linkage blocks, each only a few centiMorgans in length. These results suggest that it would be fruitful to join maps of mungbean and common bean, while knowledge of conserved genomic blocks would be useful in increasing marker density in specific genomic regions for all three genera. These comparative maps may also contribute to enhanced understanding of legume evolution.

2.4. Orthologous seed weight genes in cowpea and mungbean

Using genetic maps of mungbean and cowpea, we located major quantitative trait loci (QTL) for seed weight in both species [5]. Two unlinked genomic regions in cowpea contained QTLs for seed weight accounting for 52.7% of the variation for seed weight. In mungbean there were four unlinked genomic regions accounting for 49.7% of the variation. In both cowpea and mungbean the genomic region with the greatest effect on seed weight spanned the same RFLP markers in the same linkage order. This suggests that the QTL in this

genomic region have remained conserved through evolution. This inference is supported by the observation that a significant interaction (*i.e.* epistasis) was detected between the QTL in the conserved region and the same unlinked RFLP marker locus in both species.

2.5. Bruchid resistance in mungbean

Bruchids (genus *Callosobruchus*) are among the most destructive insect pests of mungbeans and other members of the genus,*Vigna*. Genetic resistance to bruchids was previously identified in a wild mungbean relative, TC1966. To analyze the underlying genetics, accelerate breeding, and provide a basis for map-based cloning of this gene, we mapped the TC1966 bruchid resistance gene using restriction fragment length polymorphism (RFLP) markers [6]. Fifty-eight F2 progeny from a cross between TC1966 and a susceptible mungbean cultivar were analyzed with 153 RFLP markers. Resistance mapped to a single locus on linkage group VIII, approximately 3.6 centiMorgans from the nearest RFLP marker. Because the genome of mungbean is relatively small (estimated to be between 470 and 560 million base pairs), this RFLP marker may be suitable as a starting point for chromosome walking.

2.6. Powdery mildew resistance in mungbean

We used RFLPs to map genes in mungbean that confer oligogenic resistance to the powdery mildew fungus, *Erysiphe polygoni* [7]. DNA genotypes for 172 RFLPs spanning 1570 centiMorgans of the mungbean genome were assayed in a population of 58 F2 plants. This population was derived from a cross between a moderately powdery mildew resistant (VC3890A) and a susceptible (TC1966) mungbean parent. F3 lines derived from these F2 plants were then assayed in the field for powdery mildew response and the results were compared to the RFLP genotype data, thereby identifying loci associated with powdery mildew response. Three genomic regions were found to have an effect on powdery mildew response, together explaining 58% of the total variation. At 65 days after planting, two genomic regions on linkage groups 3 and 7 were significantly associated with powdery mildew resistance. In both cases, the allele from VC3890A was associated with increased resistance. At 85 days, a third genomic region on linkage group 8 was also associated with powdery mildew response. In this case, the allele from the susceptible parent (TC1966) was the one associated with higher levels of powdery mildew resistance.

2.7. Aphid resistance in cowpea

A cross between an aphid (*Aphis craccivora*) resistant, cultivated cowpea, IT84S-2246-4, and an aphid susceptible wild cowpea, NI 963, was screened for both aphid phenotype and RFLP marker segregation [8]. One RFLP marker, bg4D9b, was found to be tightly linked to the aphid resistance gene (*Rac1*) and several flanking markers in the same linkage group (linkage group 1) were also identified. The close association of *Rac1* and RFLP bg4D9b presents a potential for positional cloning of this insect resistance gene.

2.8. Cyst nematode resistance in soybean

Several sources of soybean cyst nematode (*Heterodera glycines*; SCN) resistance have been identified in soybean. This study was conducted to identify quantitative trait loci that control disease response in three commonly used sources of SCN resistance [9]. Using genetic markers, we analyzed three segregating soybean F2 populations ['Evans' X 'Peking', Evans X Plant Introduction (PI) 90763, and Evans X PI 88788] and compared the results with those

of a previous study involving PI 209332 [10, 11]. In each case, multiple races of the nematode were tested. To uncover putative resistance loci, F2 DNA marker genotypes at between 63 and 99 loci in each population were contrasted with cyst indices averaged from 12 F2:3 progeny individuals. Four independent partial SCN resistance loci were uncovered at $P < 0.0002$ (probability per locus). One of these loci, located at the top of linkage group 'G' near RFLP locus C006V, was significant at $P < 0.0001$ in all populations and races tested. Other significant loci included one near RFLP A378H at the opposite end of linkage group 'G' from C006V, another on linkage group 'J' near marker B032V-1, and a fourth on linkage group 'N' near marker A280Hae-1. Comparisons between different SCN races indicated that some of the putative resistance loci behave in a race-specific manner. These results may serve as a resource for SCN researchers and soybean breeders by summarizing a wide range of genetic data on the soybean-SCN interaction.

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