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Molecular markers and genome mapping in cowpea

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Abstract

Molecular markers such as RFLPs exist in almost limitless number in all organisms, and these could be very useful in monitoring the loci of genes that control important traits, as well as in studying genome evolution and structure. In cowpea, a genome map based mainly on RFLP markers has been developed. This map presently has 92 markers and spans 717 cM of the genome. Using this map, quantitative trait loci (QTLs) for seed weight, pod length, and aphid resistance have been identified. Phylogenetic relationship among 44 genotypes belonging to different varieties, species, and sections in the genus *Vigna* was ascertained, following RFLP analysis. A comparison between the genomes of cowpea *(Vigna unguiculata)* and mung bean *(V. radiata)* showed that nucleotide sequences were generally conserved but entire linkage groups were not, although several large linkage blocks were still maintained by both crops.

Introduction

The availability of useful genetic markers in cowpea and other pulse crops is limited in comparison to other groups of crops. A gene list, based on a few morphological markers in cowpea, has been compiled (Fery 1985), and this list has been extended with additional markers, as reported elsewhere in this book (Fery and Singh 1997). Attempts made by researchers to confirm linkages between the identified genes have so far not yielded the desired results. Hence, the linkage orders of these identified genes (markers) have not been ascertained. From available reports, it appears that there is a need to seek additional sources of markers for developing a useful genetic linkage map of cowpea.

Molecular biological techniques provide opportunities for obtaining high frequencies of genetic markers that are useful in developing genetic linkage maps of different organisms. In addition, these molecular markers help in varietal identification and fingerprinting, genetic analysis of agronomically important characters, and in making more effective use of breeding methodologies (Beckman and Soller 1986). Molecular markers such as restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLP), and random amplified polymorphic DNAs (RAPDs) are, like other genetic markers, detected as differences in the DNA sequence of two or more individuals. A marker becomes useful when two individuals carry different forms (alleles)

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that are faithfully transmitted to the progeny resulting from a cross between them. The potential benefits of molecular markers like RFLPs, in crop improvement, have been reviewed by Beckman and Sonner (1986).

RFLP marker technology can be used to gather information about agriculturally important genes. For example, phenotypic and pleiotropic effects of genes, the number of genes influencing the character of interest, the location of genes on the chromosomes, and possibly the influence of the environment on genes can be assessed using this technology. Identification of tight linkages between genes of agricultural importance and molecular markers makes it posssible to select for the latter, thereby indirectly selecting for the genes of importance. RFLPs are ubiquitous, inherited in a Mendelian form, codominant in expression, detectable in all tissues and at all ages, and available in virtually unlimited numbers of probe × enzyme combinations.

DNA markers, especially RFLPs, have in recent times become popular in the production of genetic maps of various organisms, such as common bean (Vallejos et al. 1992), lettuce (Landry et al. 1987), rice (McCouch et al. 1988), and man (Botstein et al. 1980). Other DNA markers, such as RAPDs and variable number tandem repeats (VNTRs), have been found to be useful for generating genetic maps. Because of the potentially large number of detectable DNA markers, saturated genetic maps can be more readily obtained when these types of markers are used. A linkage map is saturated when markers are distributed ~ 5 centiMorgans (cM) apart throughout the set of chromosomes (King 1990). Genetic maps, when saturated, become more useful because all parts of the genome become accessible, thereby facilitating manipulation of individual genetic factors that are associated with traits of economic importance.

Genome mapping using DNA markers takes advantage of the large differences that exist in natural populations, and no two individual organisms are likely to be identical in their DNA base sequence. These differences may be brought about by inversions, recombination during meiosis, deletions, translocations, or transpositions. Maps developed using DNA markers can be effectively used to enhance genetic manipulations of crops and other organisms. Since the locations of these markers in the genome can be identified with a high level of precision, they can be useful for detecting genes of interest which are located near them. Sax (1923) suggested that major genes which can be scored easily should be used to identify the positions of minor genes that are of interest to the breeder. This suggestion by Sax (1923) could not be effectively put to use because identified morphological markers had large effects on phenotypes and masked the effects of linked minor genes (Tanksley et al. 1989). With the discovery of DNA markers which have no deleterious effect on plant morphology and which can be easily detected in very large numbers, those tightly linked to desirable genes can be identified and used by breeders as aids to selection.

Linkage maps based on RFLP markers are being developed for cowpea (*Vigna unguiculata*) and mung bean (*V. radiata*) using a common set of DNA clones from single copy genomic libraries of bean (*Phaseolus vulgaris*), soybean (*Glycine max*), mung bean, and cowpea (Fatokun et al. 1993a). Since a common set of probes was used for generating both maps, it is possible to carry out a comparative analysis of the two genomes (Menancio-Hautea et al. 1993). Using these maps, quantitative trait loci (QTLs) with effects on seed weight were identified on the genomes of both cowpea and mung bean (Fatokun et al. 1992). Additionally, a subset of the probes was utilized to study genetic

variability among several species in the genus *Vigna*, and this enabled the establishment of phylogenetic relationships among several accessions of the different species (Fatokun et al. 1993b).

RFLP map of cowpea

A genetic map of cowpea based on RFLP, RAPD, and some morphological attributes is being developed. The cowpea mapping population comprises 58 F_2 plants, derived from a cross between an improved cultivar IT84-2246-4 and a wild relative TVNI 963 (*V. unguiculata* ssp. *dekindtiana*). Total DNA was extracted from each F_2 plant, digested with up to seven restriction endonucleases, blotted onto nylon membranes, and probed with about 300 single-copy DNA clones derived from bean, soybean, mung bean, and cowpea. Although the two cowpea parents are known to share the same primary gene pool, partial fertility was observed in the F_1 plants. The level of polymorphism between the two cowpea parents that were crossed to generate this mapping population was found to be about 20%, which is rather low. The DNA clones that detected polymorphisms between the two parents were then used to probe the DNA of the F_2 plants. A high proportion of the probes hybridized to both parental and F_2 DNA. The low level of polymorphism observed between the two parents is usually associated with self-pollinated crops. For example, to facilitate the development of a saturated map of tomato, it was necesary to embark on interspecific hybridization to generate a mapping population (Helentjaris et al. 1988) which provided a higher level of polymorphisms than from intraspecies crosses. Like tomato, cowpea is a highly self-pollinating crop.

The cowpea genomic map now has 92 markers distributed among 85 loci (Fatokun et al. 1993a). These markers are made up of 79 genomic, 4 cDNA, 6 RAPD, 2 aphid resistance loci, and 1 seed coat texture locus. The mapmaker computer program was used to determine linkage relationships between adjacent loci and linkage order was inferred when LOD ($\log_{10}$ of the odds ratio) score exceeded 2.0. Five loci have multiple markers (12 markers). The 92 markers are distributed into 10 linkage groups, although cowpea has a chromosome number of $n = 11$. This map spans > 800 cM of the cowpea genome, implying that the mean distance between these markers is < 10 cM. Ten markers have not been linked to any of the existing linkage groups. However, effort is still being made to place more markers on this map so as to develop a saturated map for cowpea, which would facilitate the exploitation of the genetic potential of the crop.

Comparison between the genomes of cowpea and mung bean

A linkage map is also being developed for mung bean, using a similar set of heterologous RFLP markers as for cowpea. Because of the common set of RFLP markers used for both crops, it is feasible to evaluate their genomic relationship. By comparing their genomes, it should be possible to ascertain whether studies on gene action for some desirable traits in one crop can be used to infer gene action for the other crop.

It was observed that ~ 90% of the heterologous clones tested hybridized to the DNA of both crops, suggesting a high level of similarity in the nucleotide sequences of both crops (Menancio-Hautea et al. 1993). Similarly, the high level of hybridization of DNA clones from bean and soybean to the DNA of both crops suggests that all these leguminous crops, to a very large extent, share identical nucleotide sequences. In addition, 53 markers

mapped in common between cowpea and mung bean were used to verify if there were any linkage groups conserved between them. The results showed that although no entire linkage group is conserved, large blocks were retained in some of the linkage groups. Within the blocks that were conserved, the order of the loci were similar in some, whereas in others major rearrangements could be detected. This comparative analysis of the genomes of cowpea and mung bean led Menancio-Hautea et al. (1993) to conclude that insertion/deletion might have played a role in the evolution of the two crops to their respective domesticated forms.

Identification of seed-weight QTLs in cowpea and mung bean

Usually, a number of genes govern agriculturally important traits. Each gene contributes its own quota towards the expression of the trait. Such traits are quantitatively inherited. Individual contribution of the genes tends to be variable, ranging from qualitative to a vanishingly small amount. Each of the several genes affecting a quantitatively inherited trait behaves like those controlling qualitatively inherited traits with regard to the laws of segregation and recombination. Incorporating these multiple genes into varieties is usually not an easy task to accomplish, since these genes may be found in various parts of the genome. Where a linkage map is available, it should be possible to dissect these quantitative traits into discrete genetic factors, i.e., quantitative trait loci (QTLs). Using interval mapping procedures (Paterson et al. 1991a), all parts of the genome can be searched for the presence of QTLs while at the same time accurately estimating their phenotypic effects. QTL analysis allows the identification of individuals with the potential of producing progeny that will express a certain phenotype. It could also be useful in identifying those loci with small effects on the phenotype, i.e., low heritability. However, in such situations, a larger plant population will be required to detect these QTLs than in cases where the loci explain a large amount of the variation for the trait of interest.

The RFLP maps being constructed for both cowpea and mung bean were used to search for the presence of QTLs for seed weight, an important trait in both crops. The mean seed weight of each plant in the F_2 mapping population was determined. By using the computer program Mapmaker-QTL, it was possible to infer the presence of QTLs for this trait in both crops. These seed-weight QTLs, two in cowpea and four in mung bean, explained 52.7% and 49.7%, respectively, of the variation for this trait. It is noteworthy that the regions of the genomes which account for the highest amount of variation in seed weight were spanned by the same RFLP markers in them. These markers were in the same linkage order in the genomes of both crops (Fatokun et al. 1992). It was inferred from this observation that these regions of their genomes have remained conserved in the course of their evolution from the wild to the present forms. In that period, certain traits such as compact/erect plant habit, nonshattering of pods, day-neutral characteristic, and early flowering have become dominant (Smartt 1985). Since seeds represent the economically important parts in these two crops, and since large seed size is preferred by consumers, selection pressure must have been imposed in favor of higher seed weight over the years. Essentially, genes controlling seed weight are being selected along with markers associated with the trait, i.e., the nucleotide sequences of the regions with these particular QTLs for seed weight in cowpea and mung bean must have been selected by farmers growing these crops. In some annual plants, alleles of marker loci that are closely associated with

characters that determine survival and enhanced reproductive capacity tended to increase as the crops evolved from wild progenitors to the present day domesticates (Allard 1988).

The orthologous seed-weight QTLs in cowpea and in mung bean span 14.0 cM and 31.0 cM of their genomes, respectively. This observation suggests a reduction in recombination in cowpea chromosomes as compared to those of mung bean. Bonierbale et al. (1988) compared the genomes of potato and tomato, and found that frequency of recombination was significantly lower in potato. However, nuclear DNA contents of cowpea and mung bean are identical at about $2n = 1.0$ pg (Arumuganathan and Earle 1991).

Quantitiative trait loci for pod number, pod length, plant height, days to 50% flowering, and days to maturity were also detected in cowpea using data of F_2-derived F_3 (F_2:$_3$) progenies. Seed of F_2:$_3$ progenies were sown in the field at IITA, Ibadan, Nigeria, while those of the F_2 were sown in the greenhouse at the University of Minnesota, St. Paul, USA. It was noted that seed-weight QTLs detected in the F_3 population (Fig. 1) were spanned by the same markers (pA509, pO103, pA487, pM185, and pM182) as those in the F_2 population (Fatokun et al. 1992). These observations suggest that the QTLs for seed weight detected in the two regions of the cowpea genome are not particularly sensitive to environment. Paterson et al. (1991b) found that in tomato, 4 of the 29 detected QTLs were not affected by the environment. For purposes of crop improvement, QTLs that remain consistent in their effects irrespective of environment are useful, and where environment specific QTLs are available, they could as well be exploited to enhance the agricultural productivity of the crop. Combining several QTLs with different environment specificities into a genotype might induce an improvement in phenotype that is buffered against environmental vagaries (Paterson et al. 1991b).

Two loci that influence resistance to aphids were detected and mapped on the cowpea genome. One of the two genotypes (IT82 2246-4) that were crossed to obtain this mapping population is an improved aphid resistant variety. A locus that is very tightly linked to an RFLP marker (bg4D9b) on linkage group 1 was detected and a second locus, not tighly

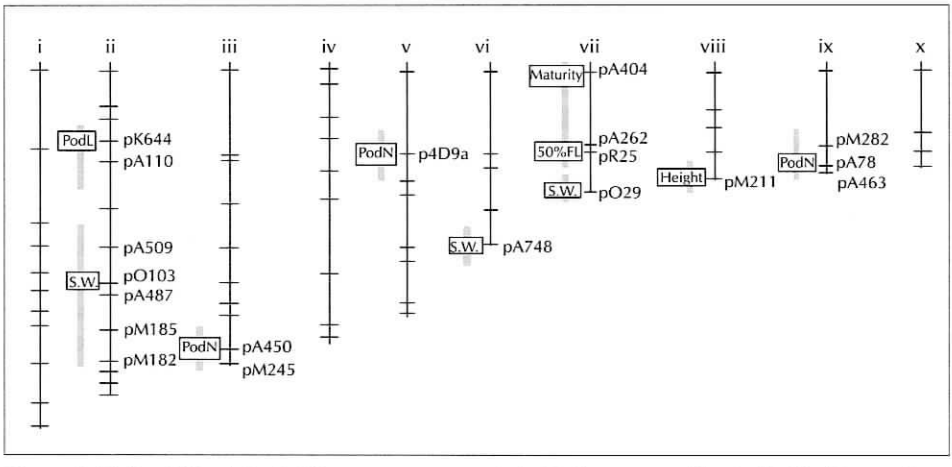

Figure 1. Major QTLs detected for some agronomic traits in cowpea. The traits (PodL = pod length, SW = 100 seed weight, PodN = pod number/plant, maturity, 50% FL = 50% flowering, and height = plant height at maturity) were measured on 58 F_2:$_3$ derived progeny rows.

linked to any of the markers mapped, is on linkage group 8 (Myers et al. 1996). Selection for the marker bg4D9b correspondingly leads to selection for one of the two loci that control aphid resistance in cowpea. Usually, screening for aphid resistance is carried out in the greenhouse or in the field during the seedling stage of cowpea. Situations exist in which plants showing resistance in the seedling stage may succumb to the pest at a later stage of growth. Markers found to be closely linked to the loci conferring resistance to aphids throughout the plant's growth stages can aid breeders in selection. Marker-assisted selection can then be carried out at the seedling stage, which should also identify resistance expressed as the plants mature.

Taxonomic relationships in the genus *Vigna*

DNA markers have been used extensively to study taxonomic relationships between and within species. Morphological attributes have traditionally been employed in establishing phylogenetic relationships among genotypes between and within species. Many of the morphological characters commonly used are prone to environmental influences, thereby reducing the fine resolution required to ascertain phylogenetic relationships. The number of morphological attributes that can be scored is generally limited. DNA markers provide a larger number of characters which are unaffected by environmental influence, and consequently can provide unambiguous character-state assignments (Sanderson and Donoghue 1989).

Morphological attributes along with cytological, phytogeographic, and crossability data have been used to study taxonomic relationships between genotypes belonging to the genera *Phaseolus* and *Vigna* (Verdcourt 1970; Marechal et al. 1981). Isozyme variations among different *Phaseolus* and *Vigna* species, subspecies, and varieties were detected by Jaaska and Jaaska (1988), but the variations were not used to evaluate relationships among the tested genotypes. However, a study on taxonomic relationships among 44 accessions belonging to several species within the genus *Vigna* has been carried out based on RFLP analysis (Fatokun et al. 1993b). All of the random genomic clones derived from soybean, common bean, cowpea, and mung bean hybridized with total genomic DNA from all of the accessions examined. This observation implies that nucleotide sequences of many of the genes are conserved in these leguminous plants so as to permit such a level of heterologous hybridization. In the Graminaea, Hulbert et al. (1990) found that maize (*Zea mays*) DNA clones hybridize very well with DNA of sorghum (*Sorghum bicolor*) genotypes; they suggested that cloned DNA fragments which hybridize to single sites in the genomes of two species can be assumed to have arisen from a single sequence in a common ancestor. Maize and sorghum are both members of the tribe Andropogonae. Cowpea, common bean, mung bean, and soybean are all members of the sub-family Papilionoideae.

For the phylogenetic study based on RFLP analysis, total DNA was extracted from each *Vigna* accession, digested with one endonuclease (*Eco*Rv), and blotted onto nylon membranes hybridized to 40 random genomic DNA clones. A few of these clones were detected as single-copy in cowpea and bambara groundnut (*V. subterranea*), but as multiple copies in mung bean and the *Phaseolus* species. An example of such is a bean genomic clone p_4D_{10}. The genotypes were not scored for any clone that showed multiple bands in any of the genotypes tested in this study. Only 27 clones were eventually scored for phylogenetic analysis. These clones gave rise to 369 RFLP bands, i.e., characters for

which each accession was scored. For each RFLP, a genotype was scored as having (1) or not having (0) a particular band. Data obtained from banding patterns were, thereafter, subjected to the NTSYS-pc program (Rohlf 1990).

The RFLP data obtained from the accessions belonging to different *Vigna* species were subjected to numerical taxonomic procedures, which showed that homology at the DNA level ranged from as low as 62% between soybean in the subtribe *Glycinae* and other test materials in the *Phaseolus-Vigna* complex to 96% between plants of the same accession in some landraces of cowpea, bambara groundnut, and mung bean. The detection of variation as low as 4% at the genome level among members of an accession further attests the robustness of the RFLP technique for genome characterization.

Morphologically, the five plants established from an accession of mung bean could not be distinguished from one another, just as the five plants from an accession of bambara groundnut also resembled each other. However, despite the morphological simililarities among members of an accession in both crops, RFLP markers were able to detect differences between the crops.

The numerical taxonomy of the genus *Vigna* based on RFLP analysis distinctly separated the genotypes into classes that were similar to those already established by conventional classification, which were based primarily on plant morphology. For example, members of sections *Catiang*, *Ceratotropis,* and *Plectotropis* were placed in their natural groups. The classification based on RFLP data also confirmed the existence of a high level of genetic variation among African *Vigna* species.

It is known that polymorphisms detected by the same RFLP probe but more than one endonuclease may not be independent mutational or DNA rearrangement events. Only one restriction endonuclease, *Eco*RV, was used to digest total genomic DNA of the various *Vigna* species tested. Hence, the polymorphisms detected in this study are independent events and the result of this numerical taxonomy should, therefore, be reliable.

DNA markers in cowpea improvement

In the process of crop improvement, the breeder manipulates the genome of the plant of interest, so that the resulting genotype meets his/her set objectives. Accomplishing the objectives will, by and large, depend on the tools at his/her disposal. In recent times, additional tools have become available to breeders in the area of molecular biology with which they can more effectively investigate, among others, the inheritance of complex desirable traits and manipulate the genetic factors associated with these traits. Using the RFLP technique, it is now possible to carry out, based on common probes, comparative studies of the genomes of organisms which cannot be crossed. The genomes of tomato and pepper, as well as those of maize and sorghum, have been compared, following development of their genomic maps based on RFLPs.

Molecular markers available in very large numbers have been found particularly useful for generating genetic maps that may help breeders in their selection work. Markers that are closely linked with traits that are difficult to score can be selected, thereby selecting indirectly for the desired trait. In such situations, selection is carried out for the marker(s) that are tightly linked to the trait of interest. Environmental factors complicate studies of the genetic control of quantitatively inherited characters, and make the selection for such traits rather difficult. Molecular markers are generally independent of environment and

their heritability values are very high. These markers can, therefore, be used to dissect quantitatively inherited traits to their simple Mendelian factors.

Cowpea is susceptible to a number of insect pests that cause considerable yield losses. Low to moderate levels of resistance have been detected in some noncultivated wild relatives of cowpea, and attempts are being made to accumulate in cultivated cowpea the genes conferring the level of resistance available. Even low levels of resistance are often controlled by many genes, i.e., they are quantitatively inherited, and may be distributed into different loci on the genomes. Partial resistance genes, where present, confer durable resistance because they pose very little selection pressure on the pests and diseases organisms (de Ponti and Mollema 1992). Therefore, plant breeders who wish to develop varieties resistant to pests and diseases should aim for this type of resistance. Marker-assisted selection should enable the genes with overlapping effects to be effectively accumulated in cultivated cowpea.

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