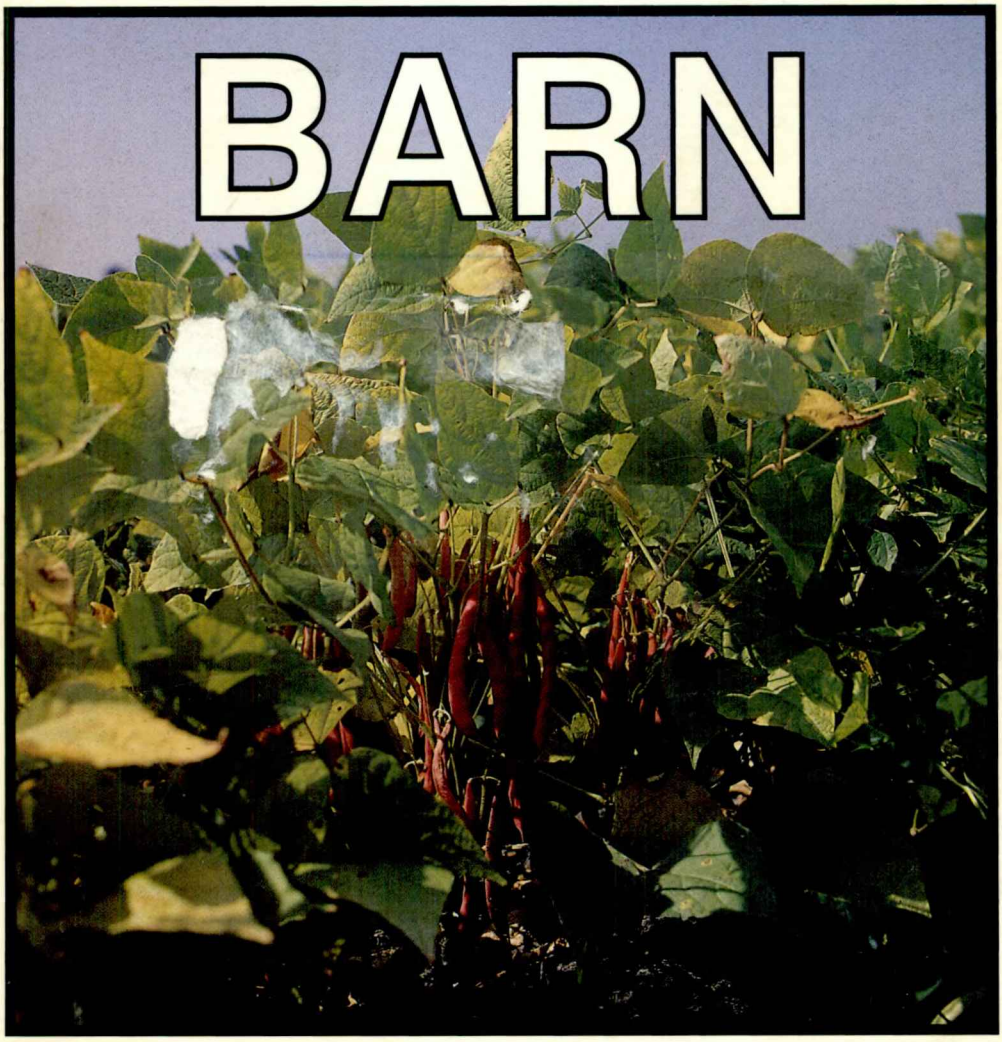
Proceedings

Phaseolus Beans Advanced Biotechnology Research Network



CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

Editors:
W. M. Roca, J. E. Mayer, M. A. Pastor-Corrales, J. Tohme

Genome structure: Uses of molecular tools

*M. J. de O. Zimmermann**

ABSTRACT

There are many possible uses of molecular techniques for plant improvement, many of which are used mostly to develop basic knowledge to help in the understanding of traits. In the case of genome studies, the main uses of molecular tools are for gene tagging, marker assisted selection and DNA fingerprinting. Gene tagging means to precisely locate genes that are of agronomical interest in the map for the crop. A natural application of such knowledge is for marker assisted selection which consists in the selection being made based on closely linked molecular markers instead on the target gene itself. The advantage of marker assisted selection is the precision with which is possible to identify a genotype without any large environmental effect since DNA is not affected by the environment: the selection is made based on presence or absence of bands. Pre requisite for a successful program is the identification of closely linked markers, and existence of polymorphisms between the parents for such markers. Marker assisted selection improves the precision of selection for the target traits, and it can be used to speed up and direct the obtention of homozygous lines with the right combination of genes, to speed up the introduction of recessive genes in backcrossing programs and to direct and improve the efficiency of pyramiding genes. Molecular markers can also be used a) to study the levels of hidden variability that might be present in some apparently uniform line, b) for fingerprinting genotypes (precisely defining genotypes), c) to follow introgressions of exotic germplasm into cultivated, d) for the construction and breakage of linkage groups (supergenes), e) for evolution studies and f) to distinguish true pleiotropy from linkages.

The understanding of genome structure in plants is receiving larger attention in the last few years. For many species, genetic maps are being made using techniques such as restriction fragment length polymorphism (RFLP), isozyme variation, random amplified polymorphic DNA (RAPDs), protein polymorphism, etc. Such maps, coupled with regular linkage studies made on morphological, agronomical and physiological traits, allow for a better understanding of the chromosomes (and of linkage groups), rates of recombination, conserved regions, correspondence or not with physical chromosome maps and genome evolution, among others.

* CNPAF-EMBRAPA, Goiânia, GO., Brazil.

For the genus *Phaseolus*, these maps started to be developed a few years ago. For *Phaseolus vulgaris*, (common bean) the most widely grown species of the genus, two maps are now available, each of them covering about 80 to 85% of the genome (Vallejos, *et al.*, 1992; Gepts *et al.*, 1993). Unfortunately, only a few morphological, physiological and agronomical characteristics are so far included in the maps. Common bean chromosomes are extremely small and it is very difficult to make cytological studies such as to relate them with the new maps based on molecular markers. The total understanding of genome structure in this species, due to such difficulties, is still far from being reached.

Even with the limitations that were mentioned, the fact that there are maps available, open a range of possible applications of that knowledge that did not exist before. Some of them are: to improve the understanding of the origins of the different gene pools within the species (Gepts & Debouck, 1991) and the possible existence of "races" (Singh *et al.*, 1991), to improve the understanding of the relationships between species in the genus *Phaseolus*, to improve the understanding of recombination among genes and the creation of new gene groups, to follow introgressions of chromosomal regions/pieces from exotic germplasm in the common bean genome, to help pyramiding genes to create improved sources of disease resistance and resistance to some environmental stresses that are responsible for complexes compound responses from the plants, and many other applications that are only limited by economical considerations and by the imagination of the researcher.

In the present paper, a few of these uses are going to be discussed: the ones that are the main objectives of the bean improvement program at CNPAF or of some of the related disciplines.

Use of molecular tools: gene tagging

There are some basic steps that need to be taken for gene tagging. For that it is understood the precise location of a gene in a linkage group of the known map. Consequently, the first step is the choice of a characteristic for gene tagging. That may be any characteristic ar any phenotype that may be precisely linked to some genotypes and environmental condition, for example a resistance or susceptibility to a known disease race, a morphology that a genotype always shows, etc.To can locate the gene or genes that determine such phenotype, as a pre-re quisite, one must have a basic linkage map for the species.

In some cases, there are characteristics that are not a single phenotype but the result of many different component phenotypes like as for Bean Golden Mosaic Virus, that some of the "resistant" genotypes show little yellowing, others show small plant distortion or small pod distortion, others show small reduction in yield but with all the other regular symptoms and so on. For these characteristics, each particular response should be treated as a different phenotype and tagged independently.

Having the map and the characteristic that was chosen, the next step is to choose parents that show contrasting phenotypes for the case and the largest number of polymorphism for the other markers in the map. After a survey of polymorphisms and parental choice, it is needed to perform traditional crosses and to obtain mapping populations which can be F2s; back crosses; recombinant inbred lines, etc. between the chosen parents.

The mapping of the characteristic is started by growing the population in numbers that are adequate for mapping and precisely genotyping each plant or line of the population almost always using the traditional procedures. The precision with which each genotype is identified makes for a large difference in the way the location of genes is achieved. Usually some sort of environmental control is recommended as well as some sort of interference to maximize the expression of some phenotypes. The plants also have their DNA extracted (some leaves only) and according to the case, either by running PCR with some chosen primers, running agarose gels and getting the pictures (RAPDS) or by cutting the DNA with restriction enzymes, running it in agarose gels, transferring to nylon membranes, labelling probes, hybridizing the DNA and performing autoradiography, (RFLPs with ^{32}P) each plant must be genotyped for the probes that they show polymorphism.

Genotyping for each probe must be as precise as it is possible, and in the case of RAPDS, the standardization of conditions must be done before any sort of study. Of course, since a map needs to be available or needs to be made, all the work (map and gene tagging) has to be done under the same conditions. A consideration to be done is that for RFLPs, usually is possible to separate without doubts the 2 homozygous classes and the heterozygous for each gene. For RAPDs, only 1 homozygous class and another that joins the other homozygous and the heterozygous are separable and that is not related to dominance/recessivity.

The genotyping can also be made for proteins and isozyme markers, following the adequate procedures for that. (Vallejos, 1983). The most markers are used, the more precise the mapping of the characteristic will be. For isozymes

and proteins, in some cases the identification of homozygous and heterozygous may be as precise as RFLPs but in other cases no, and dominance relationships may appear.

Finally, data is analyzed and gene or genes are located in precise linkage groups with known flanking markers. After gene tagging, the natural sequence is marker assisted selection.

Marker assisted selection

Marker assisted selection is the selection made based on linked flanking markers instead on the target characteristic. The advantage of it, is that in most cases, since probes are DNA markers, they exist or not and are not dependent on the environment to show their presence. In cases where isozymes or protein markers are also linked to the target gene some environmental interference may exist. Marker assisted selection is the best approach for all cases when a characteristic shows a high genotype by environment interaction or when it is a difficult characteristic to visualize or to genotype.

For this type of selection, the closer the linkage of the target gene with a probe, the better that probe is for the application of selection pressure. The basic step for marker assisted selection are: a) good parental choice; b) crossing and obtention of segregating population in adequate numbers for the characteristics that are to be combined; c) planting segregating population; d) DNA extraction of all plants; e) use subset of the population for gene tagging just to review map distances for the particular linkage group; f) use chosen procedure and probes for the particular linkage group where target gene is located and genotype all plants for the closest linked flanking markers in relation to the target genes; g) select plants based on the fact of their presenting or not the combination of flanking markers that are being sought (if possible, select homozygous plants for all flanking markers).

When the selection of only homozygous plants for the flanking markers is not possible an extra generation will be needed to obtain the right combination in homozygous state. At the end, regular trials are needed to make sure that the characteristic is being expressed as it should and also to compare with other genotypes to obtain a comparison and an evaluation of selection progress. The presence of a gene does not mean that the characteristic will show, since the environment may make some modification in its expression (heritability).

Marker assisted selection may also be used to speed up the introduction of a recessive gene by backcrossing. Since it allows the recognition of the homozygous, a generation of selfing after every 2 generations of crossing will not be needed.

Other possible uses of molecular tools for plant improvement

Besides the procedures that were described, the molecular tools that are used for mapping can also be used for studying the level of hidden variability in some apparently uniform lines (such levels are usually beneficial and correlate with yield stability); for DNA fingerprinting and achieving specific, precise identification of genotypes for registration or for characterization, to follow introgressions of exotic germplasm, for the construction and breakage of linkage groups and to distinguish true pleiotropy from linkages.

References

Gepts, P.; Nodari, R.; Tsai, S. M.; Koinange, E. M. K.; Llca, V.; Gilbertson, R. and Guzman, P. 1993. Linkage mapping in common bean. Annual Report of the Bean Improvement Cooperative. 36: XXIV-XXXVIII.

Gepts, P. and Debouck, D. 1991. Origin, domestication and evolution of the common bean (*Phaseolus vulgaris* L.). In: Common Beans. Research for Crop Improvement. Ed. Schoonhoven, A.van & Voysest, O. C.A.B. International. 7-54.

Singh, S.P.; Gepts, P. and Debouck, D.G. 1991. Races of common bean (*Phaseolus vulgaris*, Fabaceae). Economic Botany, 45:3: 379-396.

Vallejos, C.E.; Sakiyama, N.S. and Chase, C.D. 1992. A molecular marker based linkage map of *Phaseolus vulgaris* L. Genetics, 131: 733-740.

Vallejos, C.E. Enzyme Activity Staining. 1983. In: Isozymes in Plant Genetics and Breeding. Part A. Eds: Tanksley, S.D. & Orton, T.J. ELsevier, New York, 469-516.