MOLECULAR MARKER-ASSISTED BREEDING: A COFFEE PERSPECTIVE

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Introduction

Coffea arabica L. is characterised by low genetic diversity which has been attributed to the allotetraploid origin, reproductive biology and evolution process of this species (Lashermes *et al.*, 1999). However, spontaneous accessions collected in the primary centre of diversity as well as wild relative *Coffea* species constitute a valuable gene reservoir for different breeding purposes (Charrier and Eskes, 1997). Hence, transfer of desirable genes in particular for disease resistance from diploid species like *C. canephora* and *C. liberica* into tetraploid arabica cultivars without affecting quality traits has been the main objective of arabica breeding. In so doing conventional breeding methodology faces considerable difficulties. In particular, strong limitations are due to the long generation time of coffee-tree (5 years), the high cost of field trial, and the lack of accuracy of current strategy. One can estimate that a minimum of 25 years after hybridisation (five backcross-generations) is required to restore the genetic background of the recipient cultivar and there by ensure good quality of the improved variety. Combining various genes of resistance without reducing coffee quality appears therefore as a very difficult task in an acceptable time-frame through traditional breeding approaches.

In recent years, DNA-based genetic markers have gained widespread applications in many fields of plant genetics and breeding. In particular, the development of marker-assisted selection (MAS) programmes promises to overcome present limitations of conventional coffee breeding. In this report, recent molecular markers analyses as well as aspects of MAS are reviewed with regards to coffee breeding.

Molecular analysis of arabica coffee introgression lines

Occurrence of spontaneous hybrids between tetraploid arabica and other diploid species is common especially when these species are cultivated together. Exploitation of such natural tetraploid interspecific hybrids gained priority in coffee breeding and still assumes greater significance. For instance, the Timor Hybrid, a natural hybrid between *C. arabica* and *C. canephora* is being extensively used world wide, as the main source of resistance to pests and diseases. Introgressed arabica genotypes derived from the Timor Hybrid were analysed for the presence of *C. canephora* genetic material using the amplified fragment length polymorphism (AFLP) approach (Lashermes *et al.*, 2000). Although varying between the

Timor Hybrid-derived genotypes (Fig. 1), the amount of alien genetic material appeared substantial and should justify the development of adapted breeding strategies.

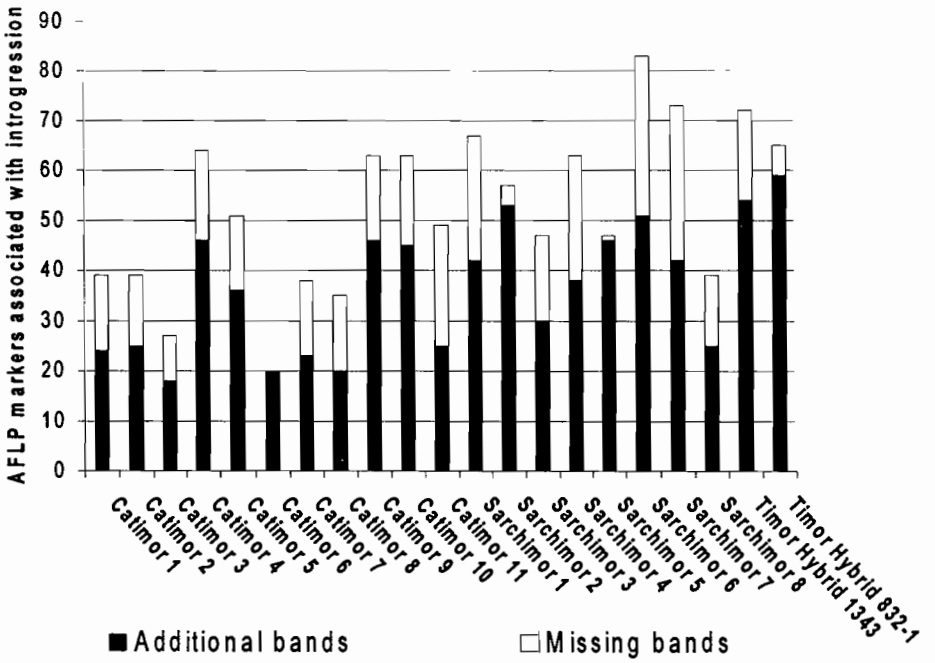

Fig. 1. Numbers of AFLP polymorphic bands attributable to introgression detected in Timor Hybrid-derived genotypes.

Selection for major genes through linkage with molecular markers

An application of molecular marker in plant breeding is based on finding tight linkages between these markers and genes of interest. Once identified, it may be much more efficient to select for the marker than for the trait itself. Regarding Arabica coffee breeding, one straightforward applications concern the introgression of pest and disease resistance genes. Benefits obtained from marker-selection depend on several factors such as the degree of linkage between the marker and the target gene, savings in time, and the relative costs of direct vs. marker-facilitated selection. However, this technology shows undoubtedly considerable interests (Melchinger 1990) for the transfer of resistance genes in a variety of circumstances such as:

* *Quarantined pathogenes*

If a virulent pathogen does not naturally occur in the test environment, artificial inoculation is prohibited for safety reasons. For instance, CBD is still restricted to the continent of Africa, and the availability of markers linked to the resistance gene(s) could allow pre-

emptive breeding in countries (Asia, Latin America) where quarantine barriers are still effective.

** Reliability/limitation of direct testing for the resistance trait*

Conventional selection progress could be hampered by the difficulty to ensure reliable test. Seedling test could also present strong inconvenient. For instance, the present test for evaluation for root-knot nematode is destructive leading to important difficulties in the utilisation of identified plant resistance sources. In addition, expression of many resistance genes can be strongly influenced by environmental conditions.

** Developmentally regulated character*

Early selection based on the marker genotype of young seedlings would be particularly beneficial for late expressed traits.

** Transfer of recessive resistance genes*

The classical procedure of transferring a recessive resistance gene includes a progeny test after each backcross generation to determine the presence of the desired allele. With MAS, the transfer can be accomplished without interruptions leading to an important time saving.

** Pyramiding of resistance genes/Combining valuable traits*

Pyramiding of resistance genes has been suggested as a strategy to provide durable resistance (i.e. coffee leaf rust). However, conventional breeding is complicated by the fact that, it is difficult or often impossible to distinguish the various resistance genotypes. Once the different genes conferring resistance to the same pathogen are tagged by tightly linked marker, they could be relatively easily be accumulated into a single genotype via marker-facilitated selection. Comparable advantages versus conventional are procured when trying to combine simultaneously resistance genes to different disease/pests.

Molecular-assisted backcross breeding

Repeated backcrossing simultaneously accomplishes two essential goals: 1) allow segregation to remove donor parent chromosomes unlinked to the target gene and 2) allow recombination to remove donor parent segments which are linked to the target gene. Both objectives could be considerably facilitated by the use of molecular markers.

Genome selection

Beside the target trait, it is important to consider the complete genome of individuals. Chromosomal segments are segregating within backcross progenies and the individuals show various contents of the desired parental genome (Fig. 2).

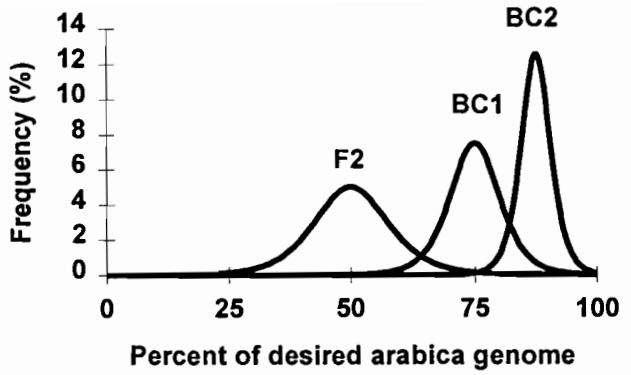

Fig. 2. Frequency of individuals in F2, BC1, and BC2 having various contents (%) of the desired parental genome.

A genome selection could therefore be performed by the use of markers scattered throughout the genome resulting in a reduction of the number of backcross generations required to restore the genetic background of the recipient cultivar. Values were estimated for a hypothetical arabica genome of 22 chromosome pairs of, on average, 100 cM each (Total genome of 2200 cM). In the absence of selection, parental donor DNA is only removed by a factor of two in each generation. Simulations are given for MAS programme in which the either 10 or 2% best (in terms of percent recurrent parent genome) individuals in each generation were used as the parent for the next generation (Fig. 3). Results equivalent to BC5 generation without selection are obtained after only two marker-assisted BC generations allowing a considerable time saving.

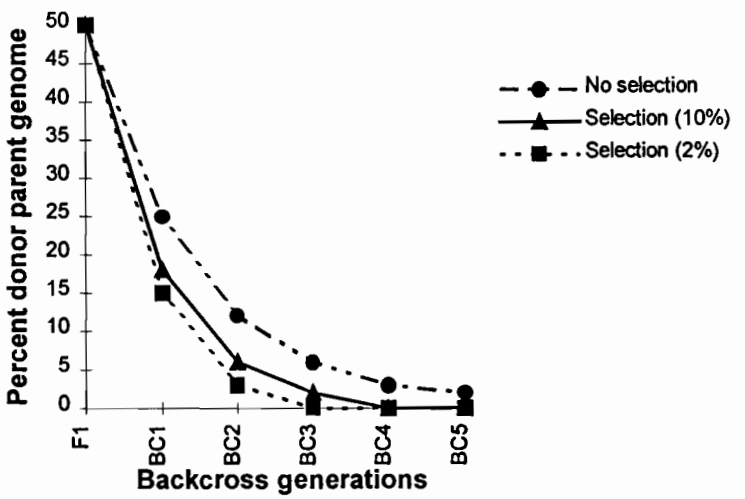

Fig. 3. Average content (%) of the donor parental genome in backcross generations under various intensities of genomic selection.

Reducing linkage drag

Removing of the linked donor segment could take many generations. Many examples of "linkage drag" are known in which undesirable traits that are closely linked to a target gene are carried out along during breeding programme. For instance in Arabica, even after 6 backcross generations, a region of 32cM flanking a target gene is expected to persist (Fig. 4). In most plant genomes 32cM is enough DNA to contain hundreds of genes. DNA markers can be used to eliminate, or at least significantly reduce, linkage drag by allowing the identification of rare recombinant individuals which are usually only selected by chance in classical breeding.

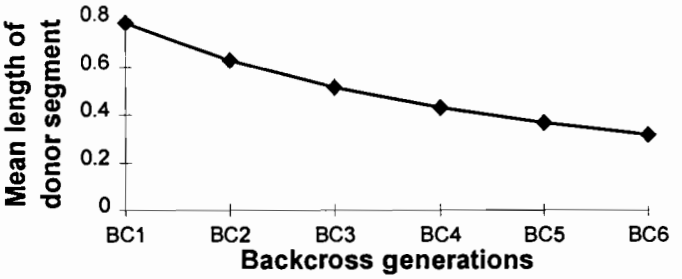

Fig. 4. Mean length of donor segment surrounding the target gene after various numbers of backcross generations. The length is expressed as a proportion of the carrier chromosome (chromosome of 100 cM long) (after Stam and Zeven 1981).

Conclusions and prospects

The development of molecular markers in coffee trees, has opened new perspectives in breeding. The conventional selection of self or back-crossed coffee-tree progenies for further breeding is extremely laborious and time-consuming. Although still requiring important efforts, the cautiously implementation of MAS is very promising (Youg, 2000). In particular, the integration of MAS in coffee, promises to drastically increase the efficiency of breeding programmes by 1) allowing for selection at an early stage and on a large number of breeding lines, 2) reducing the number of backcross cycles required to restore the quality of the traditional cultivars, 3) combining in one-step, selection for various traits or genes of resistance.

Furthermore, new findings from genome research indicate that there is tremendous genetic potential locked up in wild and cultivated germplasm resources that can be released only by shifting the paradigm from searching for phenotypes to searching for superior genes with the aid of molecular linkage maps (Tanksley & McCouch 1997). Ongoing technological developments, including automation, allele-specific diagnostics and DNA chips, will make MAS approaches based on large-scale screening much more powerful and effective. In addition, it would particularly helpful to integrate the growing body of knowledge (e.g. genomics, bioinformatics) derived from model plants (Meinke et al., 1998).

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