POTENTIAL APPLICATIONS OF MODERN BIOLOGICAL TECHNIQUES IN BREEDING FRUIT TREES

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ABSTRACT

The current methodologies of fruit tree breeding are based on segregating population and/or induced mutation, and the selection of the desired phenotype. Looking at limitations such as long juvenile period, large tree size, many not applicable technologies (backcrossing and hybrid seeds), classical breeding is expensive and very time-consuming, which makes the development of better and more efficient techniques an urgent priority. Modern biological techniques not only can be used to breed new fruit tree cultivars, but also can be applied to many other aspects of the fruit growing industry.

Modern biological techniques are not yet widely used in fruit plant breeding. One reason is that the genetics of fruit trees is still not well understood. Another reason is that reliable and efficient transformation protocols have not yet been developed for most fruit tree species.

This manuscript focuses on a few techniques which are currently available for application in fruit trees. These techniques can be used to identify important genes which can be used to generate transgenic plants. They can also be used in Marker Assisted Selection. These new genetic techniques include DNA chips and bioinformatics.

Key words: AFLP markers, SNP analysis, gene introgression, off – type plants, biodiversity

INTRODUCTION

There is no question that classical breeding techniques are currently the only ones used to produce new fruit tree cultivars. Most currently available fruit tree cultivars were developed either by randomly identifying selected seedlings or by using classic breeding techniques in traditional breeding programs. But these techniques have many limitations, which makes the development of better and more efficient techniques an urgent priority. Modern biological techniques not only can be used to breed new fruit tree cultivars, but also can be applied to many other aspects of the fruit growing industry.

Many different strategies support classical breeding and cultivation. Some transgenic plants have already been planted on a commercial scale and have made a significant impact on both current and future agriculture. Nevertheless, the real revolution has barely started, not so much because of public mistrust of genetically modified crops, but rather because the techniques currently available are still limited. Transgenic maize, rice and cotton cultivars have been quit successful. The cultivars have been modified to be resistant to herbicides, insects or viruses. Golden rice is a modified cultivar with enhanced nutritional value. However, only one transformed fruit tree cultivar is commercially available: a papaya cultivar which is resistant to PRV. One reason is that the genetics of fruit trees is still not well understood. Another reason is that reliable and efficient transformation protocols have not yet been developed for most fruit tree species.

The second way of modern breeding is development of molecular markers. DNA markers are DNA fragments that differ among the varieties of a given species. Marker profiles generated by various molecular techniques can be used to identify and distinguish cultivars and rootstocks. They can be used to enhance the efficiency of classic breeding programs. Identification by DNA markers can also be used to protect the legal rights of breeders.

A few examples of how our group has used DNA markers to improve fruit breeding and cultivation are presented below.

1. Mango malformation

Mango malformation is a devastating disease, which can cause enormous economical loss almost everywhere mangoes are grown. It is caused by the fungus *Fusarium mangiferae*. Inflorescences of infected trees are malformed and unable to bear fruit. The epidemiology of the disease is not yet well understood. No effective treatments or reliable diagnostic tools are currently available. Because the disease affects only the inflorescences, infection is extremely difficult to detect when the tree is not in bloom.

A diagnostic tool is needed to not only to keep orchards free of the pathogen, but also to better understand the epidemiology of the disease. We have developed a simple PCR based method. Using AFLP, we identified and

screened pathogen-specific DNA sequences to ensure that they did not occur either in other *Fusarium* species or in the mango genome itself. We then sequenced this fragments and designed primers for PCR. The procedure works well in the laboratory, and is being improved in terms of both specificity and sensitivity. Trials in commercial orchards are already underway.

2. Decreasing the number of generations needed for backcrossing after gene introgression

Backcrossing is a common breeding technique to introduce a specific gene from a wild variety into a commercial cultivar. Six to eight backcrosses are generally needed to dilute out the undesired wild variety genes. In species which take several years to mature, backcrossing can take decades. This drastically limits the usefulness of backcrossing in breeding fruit trees.

We have proposed using DNA marker techniques to reduce the number of backcross generations needed (Hillel et al., 1990). Even after the first backcross, the percentage of the progeny genome which is derived from the recurrent parent's genome ranges from 50 to 100%. DNA marker techniques can identify those individuals which carry the target gene and have the highest proportion of the recurrent parent genome. Depending on the number of chromosomes and the level of selection, the number of backcrosses can be reduced dramatically to one to three generations. The initial investment of screening a large number of progeny can pay back handsomely in saved time.

3. Mango

Amplified Fragment Length Polymorphism (AFLP) is a DNA finger-printing technique based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA. The technique can be used with any species and does not require any prior information on DNA sequences. Fifty to one hundred restriction fragments are typically amplified and visualized on denaturing polyacrylamide gels (Vos et al., 1995).

We have used AFLP to generate a preliminary genetic map of the mango and to assess the genetic relationships between various mango cultivars and rootstocks (Kashkush et al., 2001).

4. Date palms

Modern propagation of date palms is based on tissue culture. This technique is essential for the rapid propagation of a large number of trees and is the basis for the modern date palm industry. However, propagation by tissue culture frequently produces off-types, some of which are detected only after the plants reach maturity. This can cause major economic losses for the growers.

In order to detect and prevent off-types, propagation protocols need to be modified based on a thorough understanding of the mechanism by which off-types arise. We have been using AFLP in order to broaden our knowledge of this phenomenon.

We compared the AFLP band patterns of normal and off-type trees of the cultivars 'Barhee' and 'Medjoul'. We identified many sequence polymorphisms among the 'Medjoul' trees which had been produced by tissue culture. Nevertheless, we did not find any specific AFLP band associated with a specific off-type.

Therefore, we used methylation-sensitive and methylation-resistant restriction enzymes to compare methylation patterns in normal and off-type trees. Preliminary results suggest that each off-type has a characteristic methylation pattern. This had already been found to be the case in oil palms as well.

We are currently using cDNA-AFLP to compare differences in gene expression between normal and off-type trees. We are also studying several genes in which changes giving rise to off-types seem to occur with particular frequency. Among the changes being studied are changes in DNA sequence, methylation, and gene expression (Gurevitz et al., 2004).

5. Yeast

a. Single Nucleotide Polymorphism (SNP)

SNP's are the most common polymorphisms in the human genome and most probably in other genomes as well. Other types of sequence variation, such as chromosomal aberrations and variation in repeat number occur far less often. They do not seem to be randomly distributed throughout the genome. SNP's are under intensive investigation as potential tools to assess genetic variation and identify genes. These point mutations account for most of the genetic variation among individuals, and have thus become important tools in medical genetics and evolutionary biology. Almost 10 million human SNP's have been described and are being used in medical research, population studies and forensic science.

SNP's occur in high numbers in the genomes of all organisms. High throughput techniques have been developed to ensure rapid and accurate SNP genotyping.

b. Using SNP's to study biodiversity and hunt for genes in yeast

Saccharomyces cerevlsiae is an excellent model organism for obtaining knowledge about genetic processes which sometimes can be applied to other organisms, including fruit trees.

We have used SNP's to identify yeast genes responsible for differences in sporulation efficiency. We studied two strains of *S. cerevisiae*: SK1, which has high sporulation efficiency, and S288c, which has low sporulation

efficiency. A total of 145 genes consisting of 81,480 base pairs are known to be associated with sporulation in *S. cerevisiae*. By sequencing these genes in the SK1 genome, 554 SNP's were found which distinguished SK1 from S288c. Sporulation efficiency was determined in SK1, S288c, the F1 hybrid, and 326 diploid segregants generated from meiosis in the F1 hybrid. Sporulation efficiency was 90% in SK1, and 15% in S288c. In the hybrid, sporulation efficiency was closer to SK1 than to S228c. In the segregants, sporulation efficiency ranged from 1% to 97.5%, with an average of 69%.

Two pools of DNA were prepared: one from 21 segregants with the highest sporulation efficiencies, and the other from 21 segregants with the lowest sporulation efficiencies. Allele frequencies were calculated for each SNP in both groups by visualizing the two alleles inherited from SK1 and S288c. The only genes in which significant differences in allele frequency were found were YNL100W and RAS2, which are located 3 Kb apart on chromosome 14. This "candidate region" was found to be 175 Kb in length.

Genome-wide screening was performed by hybridizing the DNA of the two strains and the two segregant DNA pools to the S98 Affymetrix yeast chip. We identified about 4000 "probes" containing SNP's distinguishing SK1 from S288c. Regions which contained alleles which differed between the two segregant pools were also detected by hybridization. Three regions containing several probes were identified.

On the basis of the candidate gene search and the genome-wide screen, we decided to focus our attention on the "candidate region" on chromosome 14. Based on earlier research, we chose fourteen candidate genes which might play a role in sporulation or meiosis. These genes were tested to see if they contained single mutations which might account for the difference in sporulation between SK1 and S288c. Reciprocal homozygosity was also investigated. Four such genes were found.

We have verified our results using several different techniques:

1. Analysis of double and quadruple heterozygote deletions.
2. Comparative sequence analysis. A specific deletion of one A in a poly-A stretch in genes RAS2 and SWS2 was found to distinguish between SK1 and S288c. The deletion was located between the TATA box and the first codon. These findings suggest that the deletion could be the mutation responsible for the differences in sporulation efficiency between SK1 and S288c.
3. Analysis of expression patterns in the two alleles of RAS2 and SWS2 by Real Time PCR. Expression of RAS2 seems to be the same in the two alleles. However, expression of SWS2 is significantly different, especially at time zero, when there is a 2.5 fold difference.
4. Allele replacement. Allele replacement was performed using both the SK1 alleles of RAS2 and SWS2. The alleles were introduced into S288c. Background and sporulation efficiencies were measured. Sporulation efficiency was 0.7% in the S288c transformed with the decreasing allele of

the RAS2 gene, 17.1% in the wild type, and 50.1% in the S288c transformed with SWS2.

6. Concluding remarks

Our results suggest that genes controlling traits of interest in fruit trees might be identified by techniques such as searching for candidate genes and sequencing DNA pools. In order to definitively assign a function to a given gene, transformation with the specific allele has to be accompanied by a change in phenotype.

Transformation protocols have already been developed for some fruit trees and are being developed for others. Some of the methods used by human geneticists may also be of use in assigning functions to genes in fruit trees species.

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MOŻLIWOŚCI ZASTOSOWANIA TECHNIK NOWOCZESNEJ BIOLOGII W HODOWLI DRZEW OWOCOWYCH

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S T R E S Z C Z E N I E

Klasyczna hodowla roślin drzewiastych ma wiele ograniczeń. Długi okres juwenilny i wielkość roślin powoduje, że tradycyjne metody oparte na długotrwałej selekcji są czasochłonne i kosztowne. Obniżenie kosztów hodowli i jej przyspieszenie jest możliwe poprzez zastosowanie metod biotechnologicznych. Jedną z nowoczesnych strategii jest inżynieria genetyczna, umożliwiająca wprowadzenie genów kodujących pożądane cechy do genomu roślin. Technika ta nie dysponuje jednak wystarczająco wydajnymi metodykami transformacji i regeneracji roślin drzewiastych. Ograniczona jest również dostępność potencjalnych transgenów, które mogłyby sterować złożonymi procesami. W przeciwieństwie do strategii wykorzystujących inżynierię genetyczną techniki oparte lub/1 związane z analizą genomu (różnicowanie roślin na podstawie ich wzorów genetycznych, identyfikacja ważnych genów, selekcja oparta na markerach molekularnych – MAS, zastosowanie mikromacierzy i technik bioinformatycznych), są już dokładnie opracowane. Przydatność tych technik dla hodowli roślin drzewiastych została wykazana w artykule w oparciu o szczegółowe badania przeprowadzone w Volcani Centre.

Słowa kluczowe: markery AFLP, SNP, introgresja genów, bioróżnorodność