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UPOV

BMT/2/4
ORIGINAL : English
DATE : March 8, 1994

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA-PROFILING IN PARTICULAR

Second Session
Versailles, France, March 21 to 23, 1994

MOLECULAR MARKERS IN MAIZE

Document prepared by experts from France

UPOV working group on Biological and Molecular Techniques
Versailles, March 21-23, 1994

Molecular markers in maize

There is a huge amount of knowledge and data accumulated on maize and molecular markers in maize : hundreds of probes have been studied many of which are mapped and hundreds of sequences are recorded in the data bases. Many laboratories, either public or private, are trying to use these markers for their breeding programs. The purpose of this paper is to review some applications of molecular markers in the field of plant breeding and to investigate their potentialities as a tool for the characterisation of the varieties and their protection. This review is not meant to be exhaustive (which would be difficult, seeing the number of publications on the subject) but to give sufficient information as a basis of discussion on the possible use of molecular markers for varietal protection.

Maize is an outcrossing species but lines can be produced after several generations of self fertilisation. Lines are composed of genetically identical individuals. By inter crossing lines, hybrids are produced which are also homogeneous but a great deal more vigorous than their parents because of heterosis effects. Since genetic studies have pointed out that genetic vigour decreases with the genetic proximity of the parents, a lot of research work and efforts have been directed to the understanding of the bases of heterosis and for that purpose, in finding a tool for the estimation of genetic distances. Isozymes were the first molecular markers. They exhibited a high degree of polymorphism : using 21 enzymatic loci, SMITH et al. (1986) found 2.7 alleles per locus. They could also be useful for varietal identification (GOODMAN and STUBER, 1980 .CARDY and KANNENBERG, 1982 ; SMITH et al., 1986). But their usefulness in predicting heterosis was not demonstrated. No relationship was found between enzymatic distances and heterosis (HUNTER et KANNENBERG, 1971 ; PRICE et al, 1986 ; LAMKEY et al., 1987). However, enzymatic markers have some limitations :

- In practice it is difficult to reveal more than 40 enzymatic markers for a given species
- They only give information on the coding part of the genome. The main part of the DNA (more than 90%) escapes investigation.
- Their expression may vary according to the organ or to the physiological state of the plant

Therefore, emphasis was put in finding a new tool which would be able to detect differences in the DNA itself, which would not be limited in number and which would enable to study the whole genome (coding and non coding parts). The molecular biology of maize has been extensively studied over the last few years. Besides the estimation of genetic distances, other applications were looked for, like :

-characterising varieties
-constructing genetic maps
-finding linkages between markers and characters of agronomic interest

The RFLP method is the most documented and produced most molecular markers. The methods based on the PCR (RAPD, AFLP, microsatellites) appeared later and produced but a few publications.

Restriction fragment length polymorphism

RFLP have been extensively used for the study of the genetics of maize, partly because of their reliability as markers (they are codominant, they do not exhibit epistasis, their expression does not depend on the conditions of growth, stage or organ of the plant), partly because of the high polymorphism of maize (EVOLA et al., 1986). Almost all probes tested are polymorphic and the number of alleles per locus is estimated between 4.5 (GODSHALK, 1990 ; MELCHINGER, 1990) and 5.9 (LIVINI et al. ,1992). For a comparison, only about 5% of the probes are polymorphic in tomato (HELENTJARIS et al., 1989), 30% in sunflower (ZHANG pers.comm.). By sequencing homologous regions of the DNA of 7 to 9 cultivars, HELENTJARIS et al. (1989) estimate that there are 30 to 50 alterations per 1000bp in maize.

Genetic maps

Mapping markers is important not only to understand the organisation of the genetic information on the chromosomes, but also to enable an efficient choice of the markers, according to the goal looked for. Knowing the localisation of the markers allows to choose those which are concentrated on a particular region of one chromosome or, on the contrary, those which are representative of the whole genome.

Many researchers have worked on the localisation of the molecular markers of maize. HELENTJARIS et al.(1986) used monosomic lines to assign markers to particular chromosomes (maize genome contains 10 chromosomes). To locate probes more precisely on one of the arms of the chromosome, EVOLA et al. (1986) used the B/A translocations. A/A translocations may also be useful for the study of particular segments of the chromosomes. All these translocations have been maintained by and are available from the Maize genetics Cooperation Stock Center.

The first linkage map of molecular markers was reported by HELENTJARIS et al.(1986) who mapped 50 RFLP loci on 13 linkage groups. They made further effort to assign these linkage groups to the corresponding chromosomes. This map was rapidly followed by others. When common markers were available, they were generally mapped at the same position but some inconsistencies did occur due to the fact that many of the probes used were multilocus probes so that the locus displaying heterozygoty were not always the same in different crosses. As a matter of fact, HELENTJARIS and WRIGHT (1988) showed that 29% of the sequences they studied were duplicated. This is not too surprising for maize which may have an allopolyploïd origin. However they did not find homeologous chromosomes.

Immortalising the segregating populations was obtained by using Recombinant Inbred Lines (BURR and BURR, 1991): a hybrid is produced from the crossing of two genetically unrelated lines. This F1 individual is then self fertilised and produce a F2 segregating population whose individuals are each self fertilised without selection for 5 to 6 generations. Immortalised F2 populations were also obtained (GARDINER et al., 1993), which made possible to study different sets of markers on the same material. As a corollary, the same population can be used by different researchers for mapping.

The major maize RFLP mapping efforts have been made by BEAVIS and GRANT (1991), BURR and BURR, (1991), SHOEMAKER et al. (1992) and WEBER and HELENTJARIS (1989). GARDINER et al., (1993) propose a "core map" in which molecular markers are used together with isozymes and morphologic markers. This map is meant to serve as a frame for other maps and also to establish a link between maps of molecular markers and the former maps. In "Genetic maps" (O'BRIEN 1993), BURR et al. publish a map issued from a co-operative effort in which 736 markers are mapped to the 10 chromosomes of maize. Other maps are not published and belong to private societies like Pioneer HI- BRED, Native Plant Institute, Agrigenetics so that the number of mapped markers is much higher.

Linkages between markers and characters of agronomic interest

As soon as the RFLP markers became available, linkages between markers and agronomic traits were looked for. In 1991, BENTOLILA et al. found a RFLP marker closely linked to a resistance to *Helminthosporium turcicum*. Linkages with monogenic characters are relatively easy to identify. For polygenic characters, it is more difficult to find markers. GRANT et al. (1987) identified QTLs for plant height and ear height. REITER et al. (1991) found 6 QTLs for tolerance to low phosphorus stress. The behaviour of QTL has been investigated in maize by EDWARDS et al., 1987 ; STUBER et al., 1987 ; ABLER et al., 1991.

To date, there are but few example of selection gain in quantitative traits using molecular markers. LANDE et THOMPSON (1990) studied the benefits and limitations of marker-assisted breeding approaches as compared to traditional approaches. They suggest that markers may be useful for traits having a low or moderate heritability but may not improve selection for traits having a good heritability for which conventional breeding is already successful. Moreover we can expect that the marker-QTL associations obtained from a given genetic background may be lost in another genetic background.

However, the studies on linkages are useful to identify genes, to follow the introgression of a genotype into another and enable marker assisted back crosses.

Genetic distances

The estimation of genetic diversity is important not only to produce the greatest possible heterosis but also to have an efficient management of genetic resources. Therefore, classifications. of the different maize lines has often been recorded (DUBREUIL, 1992 ; DUDLEY et al., 1991 ; GALLAIS et al., 1992...). RFLP have been shown to be useful for assigning maize lines to heterotic groups (GODSHALK et al., 1990 ; MELCHINGER et al., 1990). Many research works have investigated their usefulness for predicting heterosis (LEE et al., 1989 ; BOPPENMAIER et al., 1993 ; SMITH et al., 1991 ...). The correlations between F1 performance and heterosis and genetic distances calculated from RFLP data differ according to the set of lines studied (CHARCOSSET, 1992). When the sample includes close lines, the correlation is found significant. Otherwise, when only lines from different heterotic groups are crossed, the correlation is low.

A high correlation between RFLP distances and pedigree is expected if random mating and no selection pressure are assumed which is not very likely in selected lines. However, SMITH et al. (1991) have been able to find high correlations between the RFLP distance, the pedigree and the yield heterosis. MESSMER et al. (1993) also find a high correlation between the genetic similarity based on RFLP data and the coefficient of coancestry. They think that RFLP-based estimates reflect more the true genetic similarity than does the coefficient of coancestry provided more than 100 probe-enzyme combinations are used.

Estimates of the correlations between RFLP distances and morphologic distances have also been made. A French study conducted on 150 lines described with 82 RFLP probes combined with 3 restriction enzymes, shows a correlation of only 0.22 to 0.3 between RFLP distances and morphologic distances (BAR HEN pers. comm). All the lines estimated different by the experts were indeed different from the RFLP data. Conversely, high RFLP distances were sometimes associated with a small "expert distance". This could have been expected since the objectives of selection are often the same for different breeders.

The estimation of a genetic distance can have important implications in seed trade legislation. SMITH et al., (1991a) show that the closest American varieties are much closer to each other than are the closest French varieties. SMITH et al., (1991b) make a proposal for the homologation of new varieties from which a maximum authorised similarity between the

parental lines of a new variety and the known lines could be set, based on the estimation of the RFLP distance.

OTHER METHODS

PCR based methods are now becoming more and more commonly used. This can be explained by the relative ease of use of the method, short delay for obtaining the results, possible non radio-active detection, important polymorphism detected, possibility of automation...

Although these types of methods have considerably been developed over the last few years in many cultivated species, maize geneticists have not published much about the subject. There are many explanations to this situation :

The RFLP markers are so informative that there is no crucial need for new types of markers

Most laboratories involved in maize research have invested much time and money in the equipment for RFLP. Once the apparatus is acquired, none of the two methods appears to be the most cost and time efficient over all ranges of sample sizes and number of marker loci studied (RAGOT et HOISINGTON, 1993).

Actually, many private laboratories do use PCR based methods but have not published their results.

At present there exists but a few publications in maize on these methods :

Random Amplified Polymorphic DNAs (RAPD)

This type of marker is very polymorphic (WILLIAMS et al., 1990 ; WELSH and Mc CLELLAND, 1990) but generally dominant. Their repeatability requires strict experimental conditions and great care during the manipulations. In maize, WELSH et al. (1991) succeeded in identifying inbred lines and their hybrids by RAPD markers. They suggest that 3 or 4 polymorphic primers would be enough to allow an efficient parentage determination of hybrids. HEUN and HELENTJARIS (1993) studied the segregation of RAPD markers in progenies of 16 hybrids involving 5 genetically distinct parental lines. They showed that more than 90% of the polymorphic fragment were inherited as expected. Those that were not, repeatedly exhibited the same aberrant behaviour across the different progenies.

RAPD can be transformed into codominant markers by sequencing particular DNA bands, amplifying them and making a digestion of the amplification product. This technique is called SCAR (Sequenced Characterised Amplified Region) but has not been used in maize.

Amplified Fragment Length Polymorphism (AFLP)

AFLP strategy was developed by ZABEAU and VOS and is now patented. More difficult to obtain, AFLP markers seem to be highly polymorphic and more repeatable than RAPD and gives much more information in one banding pattern. Although ZABEAU declares that AFLP are codominant markers in some cases, since image processing can distinguish heterozygotes from homozygotes by the intensity of the bands, this may be difficult. SMITH et al. (pers. comm., MGNL 1994) described 35 lines with RFLP, AP-PCR (arbitrarily primed PCR) and AFLP and found that the data obtained from the different methods were highly intercorrelated and that the different cluster analysis gave similar results. They conclude that

any of these methods can provide reliable information for varietal identification and breeding so that technological choice can be made according to the circumstances.

Microsatellites

SENIOR and HEUN (1993) have searched the sequence data bases for microsatellites and found 6 microsatellite sequences among 280 sequences recorded in maize. They were all in enzyme sequences and polymorphic. Using 8 different inbred lines, 3.5 polymorphic patterns were generated per microsatellite. These markers were shown to be suitable for maize mapping.

All these publications on PCR based methods sound optimistic, but none of them involves enough markers or enough lines to enable a comparison of their potentiality with that of RFLP markers. However, it can be expected, from the amount of work now undertaken in many laboratories on microsatellites, that this technique will be much developed in the next few years. As a matter of fact, among the PCR derived methods, microsatellites seem the most suitable for genetic studies and for mapping. Besides, it is possible that RFLP markers will be adapted to PCR technology ,for example, by sequencing their extremities, producing the corresponding primers and looking for polymorphism inside the amplification product. These markers, not yet documented in maize, are called CAPS (Characterised Amplified Polymorphic Sequences). They are codominant like RFLP markers and easy to screen but may be less polymorphic.

Conclusion

Maize is one of the most economically important crops and is among the best known species. Its genetics has been extensively studied and it can be considered as a model crop plant for molecular marking. Moreover, different genetic materials exist and are available : lines, hybrids, monosomics, A/A and B/A translocations... All molecular methods used so far have proved to be efficient for varietal identification and estimation of genetic distances. If these tools are to be used in homologation schemes or, more likely, for the study of essential derivation, the data are available and ready to use.

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