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In English only

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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

TECHNICAL COMMITTEE

Twenty - eighth Session
Geneva, October 21 to 23, 1992

AFLP:
A PRACTICAL SOLUTION TO MEASUREMENT OF GENETIC DISTANCE AND DEPENDENCY ISSUES

Information received by the Office of the Union

(Original)

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October 6th 1992

Dear Barry

I enclose herewith copies which I feel may be of interest to you and your colleagues. We circulated leading national breeder associations and all officers of Assinsel with this information and it struck me it could helpful for you to be in the picture.

Meetings have been, or soon are to be, held with testing authorities in the Netherlands and UK etc. I hope the enclosures are largely self-explanatory but if you need more please don't hesitate to let me know. Meantime, I hope all is well with you all.

Best regards.

Yours ever.

T.Martin Clucas Martin

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October 1992

AFLP: A Practical Solution to measurement of Genetic Distance & Dependency Issues

I am writing to you in your capacity as President of Assinsel. Whilst, no longer, involved directly in the work of Assinsel, I am very concious that Dependency, and its associated issues of Essential Derivation and Minimum Distance, is the key topic of current debate in the industry. Furthermore, my activities over the last two years have brought me close to Biotechnology research and made me aware of recent useful progress.

One development, in particular, strikes me as having a practical role to play in routine measurement of "Genetic" Distance at an affordable cost. The purpose of this letter, which I have also sent to the President and Crop Section Chairmen etc is simply to alert you to this technology so that it can be considered alongside other pathways which may be under consideration.

It has been clear for some time that RFLPs, RAPDs and other PCR based techniques would, from the technical perspective at least, make a relevant contribution to measurement within the context of "genetic" distance. However, the limitation of existing techniques in terms of utility, adaptability to a range of species, speed and cost have been a constraint to the routine useage of such methods in many crops. In the last twelve months a new method, Amplified Fragment Length Polymorphism (AFLP), has been developing rapidly and has now reached the stage where it is being used successfully in commercial breeding programmes in an increasing range of, but not all, crops. Furthermore, AFLPs show every prospect of being invaluable in the measurement of "genetic" distance and consequently in the whole complex issue of Dependency and the clear identification of individual lines, varieties and hybrids. The AFLP technique is more reliable, flexible and powerful; hence it allows more precise measurement of "genetic distance" at a greater speed and lower cost.

A detailed description of the technique, with particular regard to its use in the measurement of "genetic" distance, is attached. The author, Dr. Marc Zabeau, is Managing Director of Keygene the biotechnology company which developed the technology. I am in no doubt that Dr Zabeau would be pleased to give a presentation of the technique to Assinsel Committees and Working Groups if this was felt useful. Equally, a group of Assinsel crop, legal and other specialists would be welcome to visit the Keygene laboratories in Wageningen.

In order that there should be no misunderstanding, I would advise you that I am assisting Keygene with the commercial exploitation of technology developed by the company. Thus we are also drawing the attention of "official circles" to the perceived value of the technique within the specific context of Minimum Distance. If I can assist further in any way please don't hesitate to let me know. Warmest regards.

Yours sincerely,

T.Martin Clucas

AFLP: AMPLIFIED FRAGMENT LENGTH POLYMORPHISM

A novel technique for DNA Fingerprinting & measuring Genetic Distance

1. THE PRINCIPLE OF AFLP

AFLP is a PCR based technique in which small restriction fragments, obtained by cleaving genomic DNAs with restriction enzymes are amplified to produce simple DNA fragment patterns. The rationale of the AFLP technique is based on the use of specifically designed PCR primers which selectively amplify a small subset of restriction fragments out of a complex mixture comprising as many as several million different fragments. The method yields DNA fingerprints the complexity of which can be programmed by the choice of restriction enzymes and primers. The products of the reaction can be visualised by conventional DNA staining or labelling procedures using both radioactive and, also, non-radioactive methods.

2. AFLP FINGERPRINTING OF PLANT DNA

The AFLP technique has been used successfully to fingerprint DNA from a broad range of crop plants including: Tomato, Hybrid Corn, Potato, Pepper, Lettuce, Cucumber, Oilseed Rape and Sugar Beet. Typically, the PCR products are radioactively labelled and fractionated on high resolution denaturing polyacrylomide gels. These AFLP fingerprints display 50 to 100 bands. The fingerprints are repeatable both in terms banding patterns and the relative intensity of each band. Most of the bands have been shown to be derived from unique restriction fragments. The quantitative nature of the amplification is well illustrated by the perfect correlation between band intensity and restriction fragment copy number: homozygous bands will be twice as intense as heterzygous bands. The AFLP technique provides a highly consistent and very precise method for DNA fingerprinting which is broadly applicable to most crop plants.

3. RESTRICTION FRAGMENT POLYMORPHISMS

When comparing AFLP fingerprints of lines or varieties one can identify, a) polymorphic restriction fragments which are present in certain lines but not in others and, b) non-polymorphic restriction fragments present in all lines. The ratio of polymophic to non-polymorphic bands varies from crop to crop and, furthermore, the frequency of AFLP polymorphisms closely match that found using conventional RFLP analysis. Typically, Hybrid Corn lines will display a high degree of polymorphism ie, up to 50% of the bands are polymorphic; by contrast, other crops, including Tomato,

Pepper and Cucumber exhibit a much lower level of polymorphism of 2% or less. Crops such as Lettuce, Oilseed Rape and Sugar Beet show intermediate polymorphism frequency. Molecular genetic analysis has shown that restriction fragment polymorphisms are due to mutational alterations including, point mutations affecting restriction enzyme cleavage sites or insertions and deletions. AFLP fingerprinting of segregating F2 populations have shown that most of the polymorphic restriction fragments exhibit Mendelian segregation ratios.

4. COMPARISON WITH OTHER DNA FINGERPRINTING TECHNIQUES

The advantage of the AFLP technique over conventional methodology lies in both speed and efficacy: in a single AFLP reaction 50 to 100 restriction fragments are visualised as against only one, or perhaps a few at most, in the case of RFLP analysis. Moreover, the PCR based AFLP reaction requires much less starting material and is less labour intensive. These advantages translate into a much lower cost for the same amount of information. Furthermore, AFLP rquires no probe collection and can be used on any plant species with out prior knowledge. Finally, AFLP also compares favourably with other PCR based fingerprinting techniques in both its high resolution and repeatability; this being due to the fact that AFLP is based on a better design of the PCR reaction.

5. VARIETY IDENTIFICATION AND ANALYSIS OF GENETIC DISTANCE

Because of its high resolution, consistency, accuracy and scope of application, AFLP fingerprinting is ideally suited for varietal identification. Several pilot studies have been conducted in which AFLP fingerprinting was used to measure genetic distance between commercial varieties of Hybrid Corn, Tomato, Lettuce, Cucumber and Oilseed Rape. Although the number of differences found between lines varied considerably between crops, in every case differences were detected which correlated well with known genetic distance. In instances where differences were very infrequent, several fingerprints were made of each line in which one or more thousand restriction fragments were scored. This data supports the conclusion that the AFLP technique out-performs others, in terms of accuracy, speed and cost-effectiveness, in measuring Genetic Distance in a broad range of crops.

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