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374

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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

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

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EVALUATION OF THE POTENTIAL OF AFLP TOWARD THE STUDY OF DISTINCTNESS IN RICE CLOSELY RELATED VARIETIES AND THE USE OF RFLP FOR VARIETY TESTING IN RICE

Document prepared by experts from Japan

375

EVALUATION OF THE POTENTIAL OF AFLP TOWARD THE STUDY OF DISTINCTNESS IN RICE CLOSELY RELATED VARIETIES

Rice (*Oryza sativa*) has been considered as one of the most important agricultural species and has been analysed genetically very well as a model plant of molecular and genetic studies. Most genetic analyses have been carries out with *indica/japonica* crosses, due to the low polymorphism frequency of DNA markers between *japonica* varieties. However, Japanese rice breeding work is mainly based on crosses *of japonica* varieties and some elite varieties are closely related each other. To perform analysis for the distinction of such closely related varieties effectively and conveniently, an application of DNA polymorphism analysis which is able to scan whole genome at a time, is desirable. We have started to investigate differences of closely related *japonica* varieties using AFLP (Amplified Fragment Length Polymorphism).

As a primary study, we have evaluated the potential of AFLP for the detecting polymorphism and its stability, two closely related *japonica* varieties of rice, Nipponbare, Koshihikari and one *indica* variety Kasalath, were used. The fluorescence AFLP Plant Mapping Kit (PE Applied Biosystems) and computer software to analyse detected fragments were used to perform AFLP analysis. To evaluate the ability of detecting polymorphism, analysis was performed with 64 combinations of primer. To evaluate the stability, one combination of primer and DNA samples extracted from the same variety but different individuals and different year, were used: 5, 2 and 3 DNA samples of Nipponbare, Koshihikari and Kasalath, respectively. Moreover, each DNA samples were divided into 3 test samples, then 30 samples were analysed at a time. From the result of polymorphism test, 47 to 140 DNA fragments ranged from 50 bases to 400 bases were detected on each variety and primer combination. Depend on the primer combination, zero to 3 polymorphic bands were detected between Nipponbare and Koshihikari, whereas 17 to 28 between Nipponbare and Kasalath. From the results of stability test, polymorphic bands were detected only between samples derived from deferent varieties, no polymorphic bands were detected in those from the same variety.

These results suggest the effectiveness of AFLP technic for detecting polymorphism between closely related varieties of rice.

Further analysis using 15 *japonica* rice varieties are under examination and will be reported in this meeting.

THE USE OF RFLP FOR VARIETY TESTING IN RICE

Rice is a very important staple food crop and has been analysed genetically well as a model plant of molecular and genetic studies. So far, studies of distinctness in rice have been carried out using cross compatibility, fertility of crossed generation, biochemical characters such as isozymes and so on. As a result, *Oryza sativa* which is a cultivar in Asia, has been classified in some ecotypes associated with its geographical distribution. However, studies of detailed classification for rice varieties belonging to the ecotype, *japonica,* which are mainly used as genetic resources in Japanese breeding program, have not been carried out due to the difficulty of detecting genetic differences.

We investigated the frequency of RFLP between *japonica* rice varieties to examine the possibility of DUS testing in *japonica* rice varieties. Here, we introduce a trial study of classification and distinctness in closely related *japonica* rice varieties using RFLP. Fifteen *japonica* varieties (10 landrace and 5 elite varieties) and 1 *indica* variety were analysed. Eight kinds of restriction enzyme were used to detect RFLPS. We have constructed a high-density linkage map using an F_2 population derived from Nipponbare (*japonica*) and Kasalath (*indica*) and over 2300 RFLP markers. These RFLP markers were randomly selected as probes for RFLP analysis. Five hundred and five probes were analysed. The number of DNA clones which detected RFLP between *japonica* varieties ranged from 38 (7.5%) to 89 (17.6%) depend on the varietal combination, whereas over 80% of the clones detected RFLPs between an *indica* and the *15 japonica* varieties. These analysis made it possible to show chromosomal uniformity in *japonica* varieties. High uniformity was observed on chromosome 5. Cluster analysis (UPGMA) was carried out using 185 RFLP bands from 73 probes. Four elite varieties out of 5 were separated from landrace varieties.

Frequencies of RFLP between *japonica* varieties were relatively low. However, presence of some polymorphism among the *japonica* varieties suggested the effectiveness of RFLP for the studies of classification in rice *japonica* varieties. The use of DNA-based markers is thought to make more detailed DUS testing feasible effectively. Other kinds of markers, such as RAPD, AFLP and SSR markers, will be required to DUS testing more effectively.

Further study to evaluate the potential of AFLP for DUS testing is under examination.

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