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PROJECT FOR PRESERVING SPECIMENS AND DNA OF PROTECTED VARIETIES IN JAPAN

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PROJECT FOR PRESERVING SPECIMEN AND DNA OF PROTECTED VARIETIES IN JAPAN

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Situation in Japan

1. Since the number of applications, registrations and consultations for infringement on Plant Variety Protection (PVP) system has recently been increasing, it is desired to conduct DUS test effectively, and to solve urgently infringements on Plant Breeder's Right (PBR).

2. Under these circumstances, the Plant Variety Protection and Seed Act was revised and breeder's rights were strengthened during the period from 2003 to 2007. DNA-based variety identification techniques ("DNA techniques") have become expected methods both for PBR holders and persons under suspicion of infringing PBRs, because of their rapidity and precision for identifying many varieties.

3. It is very important to use reference varieties of which the originality and background of breeding are clear for developing DNA techniques. Presently, the preservation of standard samples that can be applied for DNA techniques are being given attention in Japan.

Necessity of DNA techniques and samples for PVP

4. There are not many examples in which DNA techniques have been used for cases of infringement of PBR in Japan, however, it is very effective to use DNA techniques for the immediate resolution of disputes over infringement of PBR. PBR holders expect to use DNA techniques in such cases because of the long time required to conduct DUS growing tests, the difficulty to obtain seeds and seedlings of infringing varieties for a similarity test, and the difficulty to reproduce a plant from a part of infringing materials.

5. Therefore, it is desired to develop DNA techniques for variety identification which can be conducted in a short time with high repeatability.

6. If we estimate the reliability of developed DNA techniques, it is very important to use standard samples for which the originality and background of breeding are clear. Samples used for DNA techniques in PVP should be prepared systematically and quickly. In practice, samples for DNA identification test are offered by the PBR holders and/or persons under suspicion of infringing PBRs. In addition, we have to believe the samples provided by the PBR holders are genuine samples of the varieties (the original varieties for which protection has been granted). Therefore, it is necessary to establish a system where neutral organizations (such as the NCSS) preserve and, on demand, provide original plants that have the same characteristics as at the time of the grant of PBR.

Effort for establishment of consensus of DNA techniques and samples

7. It is necessary to establish a consensus on the DNA techniques and samples. It is also important to develop an understanding of the practical use and system of DNA techniques and samples by the persons involved in PVP. In Japan, some efforts to gain consensus have been made.

8. Concretely, the following four points have been addressed:

a) Acceleration of developing DNA markers and techniques.
b) Information disclosure of DNA techniques and results of the validation of reproducibility when using those DNA techniques.
c) Public awareness of basic contents that should be noted for the developed DNA techniques, and
d) Construction of a system for preserving DNA samples of PBR protected varieties and reference collections by the approval of PBR.

9. The Ministry of Agriculture, Forestry and Fisheries in Japan (MAFF) developed a guideline in the development of DNA techniques and practical use, and showed the matters that had to be noted for the identification of plant varieties by using DNA in 2003 (*http://www.hinsyu.maff.go.jp/hogo/dna_manual/pdf/guideline.pdf*). In NCSS, the Guideline for method validation of DNA identification protocols was finalized in 2008 (*http://www.ncss.go.jp/main/DNA/DNAguideline.pdf*). Information on DNA techniques and samples has been published on the website.

10. MAFF started a new program for validation of repeatability in DNA techniques and the preservation of specimens and DNA of protected varieties in 2008 in order to strengthen PBR and to defend intellectual property. NCSS has been preserving specimens and DNA of protected varieties from June 2008, within the budget of MAFF. The development of DNA markers and techniques has been accelerated and the DNA techniques developed have been validated. At the same time, the importance of the preservation of reference varieties has risen.

11. Presently, there is a lot of information regarding DNA markers and techniques. Information on advantages, ability of analysis and aspects that should be noted in using DNA techniques is available. In the preservation of specimens and DNA, samples of protected varieties are preserved with the approval of PBR holders. A mutual understanding of DNA techniques and the importance of samples provided by PBR holders has been obtained.

Project for preserving specimens and DNA

12. NCSS is collecting and preserving samples. Three types of samples are preserved: specimens, freeze-dried leaves and isolated DNA. NCSS can offer samples in accordance with the Rules for Preservation and Use, and with the agreement by PBR holders. The Rules for Preservation and Use were developed by a committee composed of lawyers, researchers, representatives of PBR holders, specialists regarding preservation, private companies, NCSS and MAFF in 2009, and are available to the public on the website of NCSS. (*http://www.ncss.go.jp/main/DNA/DNAhozon.html*).

Method

13. In the isolation of DNA from samples, the Genomic-tip method (Yamamoto *et al.*, 2006) was used. Homogenized tissue powder was suspended in 10-15 ml of G2 extraction buffer (0.8 M guanidine-HCl, 30 mM EDTA, 30 mM Tris-HCl, pH 8, 5% Tween-20, 0.5% Triton X-100), 4 µl of RNase solution (100 mg / ml), 0.2 ml of 2-mercaptoetanol and 100 mg polyclar AT, and then incubated at 50 °C for 2 hours. The suspension solution was added to Genomic-tip20/G and washed twice with 2 ml of QC buffer. Genomic DNA was eluted with 1 ml of QF buffer and precipitated with ice-cold 2-propanol. The recovered DNA

was dissolved in 100 µl of TE buffer. Isolated DNA was estimated the concentration, degree of purification and PCR amplification. After estimation of isolated DNA, it was divided into tubes and dried by using a vacuum freeze dryer, and then incubated at -80 °C in a freezer.

Perspectives of DNA samples for PVP

14. DNA techniques are an indispensable and important tool for PVP. A database consisting of DNA profiles, morphological data and photographs of varieties, is very useful for the immediate resolution of disputes over the infringement of PBR.

15. It will be necessary to collect and preserve many more samples of reference varieties and protected varieties in order to increase the applicability of DNA techniques for variety identification.

16. Now, we think that cooperation with other countries will help to accelerate the development of DNA techniques and help with the immediate resolution of disputes over the infringement on PBR. We hope to construct a practical system for the development of DNA techniques, for resolution of disputes on PBR, for information disclosure and to offer appropriate samples in the world in order to strengthen PVP.

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