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

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ADDENDUM

PROJECT FOR PRESERVING SPECIMENS AND DNA OF PROTECTED VARIETIES IN JAPAN

Document prepared by experts from Japan
The Preservation of specimen and DNA from protected varieties

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Ottawa, May 11-13, 2010

Current status of increasing demand for reinforcement of PVP in Japan

- Increasing the number of applications and registrations.
- Increasing the number of consultation for infringement cases.
- Strengthening of breeder’s rights by the revision of the seeds and seedlings law during the period from 2003-2007.
**Importance of sample preservation (1)**

**Estimation of the reliability of DNA techniques**

- It is necessary to use varieties which originality and background of breeding are clear for it. For example, originals of granted varieties and standard varieties.

**Practical use of DNA techniques in Japan**

<table>
<thead>
<tr>
<th>Crops (processed foods)</th>
<th>DNA analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>strawberry</td>
<td>CAPS</td>
</tr>
<tr>
<td>kidney bean</td>
<td>RAPD-STS</td>
</tr>
<tr>
<td>azuki bean</td>
<td>RAPD-STS, SSR(SCR)</td>
</tr>
<tr>
<td>(azuki bean jam)</td>
<td>SSR</td>
</tr>
<tr>
<td>rice</td>
<td>SSR</td>
</tr>
<tr>
<td>(boiled rice)</td>
<td>CAPS</td>
</tr>
<tr>
<td>rush</td>
<td>SSR</td>
</tr>
<tr>
<td>tea</td>
<td>SSR</td>
</tr>
<tr>
<td>sweet cherry</td>
<td>SSR</td>
</tr>
<tr>
<td>Japanese pear</td>
<td>SSR</td>
</tr>
</tbody>
</table>

(URL: http://www.hinsyu.maff.go.jp/)

**Varieties under development of DNA techniques**

- Carnation
- Cabbage
- Chinese cabbage
- Egg plant
- Chrysanthemum
- Shiba, Soybean
- Cymbidium
- Apple, Peach
- Others

**Importance of sample preservation (2)**

**Urgent solution for violation dispute**

- Plants of protected varieties are immediately necessary.
- Provided varieties must be maintained characteristics that do not change from granted time.

*when the infringement of PBR occurred,*

- The maintenance and regeneration of original plants were entrusted to PBRs till now.
- Characteristics of protected varieties must be maintained for a registration period.
Efforts to gain consensus about DNA techniques and samples

- Acceleration for developing of DNA techniques
- Information disclosure of DNA-tech and samples
- Public awareness of basic contents that developed DNA techniques could be used practically, and
- Construction of the system of preserving samples

Approach conducted for gaining consensus of DNA techniques and samples

Three kinds of guidelines

1. The development of DNA techniques and practical use in 2003 by MAFF.

2. Guideline for method validation of DNA identification protocols in 2008 by NCSS
   (http://www.ncss.go.jp/main/DNA/DNAguideline.pdf)

3. The rules for the preserving and use in 2009 by NCSS
   (http://www.ncss.go.jp/main/DNA/DNAhozon.html)
Preservation of specimens and DNAs

The project for preserving specimens and DNAs started from June, 2008 at NCSS within the budget of MAFF

Strengthening
- More promotion of the DNA technology development

**Principle**

- **Enforcement period:** 2008/6/4 - 2011/3/31
- Kinds of plants
  - Granted varieties in plants of vegetative propagation (except for seeds and strains)

**Contents**

Samples are preserved and used by the approval of PBR holders.

**Expecting effects**

More than 90% of granted varieties.

Seeds and strains are preserved at the time of appliance in NCSS.

Plants of vegetative propagation are preserved by only the person of PBRs

Plants of vegetative propagation

Mushrooms

Seed propagation

**How to preserve**

**Three types of samples**

- **Specimen**
  - Full bloom stage

  In order to keep the originality, PVP G-men collect after the completion of DUS test

- **Freeze-dried leaves**

  Leaves of granted varieties are collected and submitted by PBR holders

- **DNA (–80°C)**
  - Genomic-tip20/G method (QIAGEN)

  Genomic DNA is extracted and preserved in deep-freezer from the only plants when varieties identification is possible by DNA techniques developed
Procedure of making pressed specimen

- Samples
- Arranging
- Pressing
- Drying
- Extermination of insects (-30°C)

Example of freeze dried leaves samples

- Leaves entered in an envelope.
- Freezer (–80°C)
- Vacuum freeze dryer
- Dryness shelf with controlled moisture and temperature
DNA preservation

Yamamoto et al. (2006)
Breeding Science
56: 165-171

Example of DNA tube

Freezer (-80 °C)

No. of varieties preserved in NCSS

<table>
<thead>
<tr>
<th>Plant name</th>
<th>No. of varieties</th>
<th>2010/3/10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chrysanthemum rose</td>
<td>219</td>
<td></td>
</tr>
<tr>
<td>carnation</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>strawberry</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Citrus</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Calibrachoa</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>petunia</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>peach</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>others</td>
<td>834</td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>1,572</td>
<td></td>
</tr>
</tbody>
</table>

Samples are managed with a computer

Example of DNA tube
**Perspectives on DNA samples for PVP**

DNA techniques is one of indispensable tools for PVP.

More development of DNA-based variety identification techniques (SSR, SNP).

- **database**
  - DNA profiles.
  - No. of varieties.
  - No. of markers.
  - Morphological data.
  - Pictures of varieties.

Many more samples of reference varieties and protected varieties

Many more samples of granted varieties

cooperation with other countries

Protection of PBR

- Verification of the infringement of PBRs
- Immediate resolution of disputes over the infringement of PBR
- Propagation and maintenance of reference collections

**Acknowledgements**

Members of the examination committee in the project for preserving specimen and DNA

- Tsukuba university
- National Institute of Floricultural Science
- National Institute of Agrobiological Sciences
- National Institute for Agro-Environmental Sciences
- Law office

(in addition to some nursery companies)

**NIFTS**  Dr. T. Yamamoto

**NCSS**  Dr. K. Maruyama,
Members of Plant Varieties Protection Division and DUS Test Division

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