



BMT/14/6 Add. Rev.

ORIGINAL: English

DATE: November 27, 2014

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

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

**Fourteenth Session
Seoul, Republic of Korea, November 10 to 13, 2014**

ADDENDUM TO DOCUMENT BMT/14/6

USE OF DNA VARIETY IDENTIFICATION TECHNIQUE FOR MEASURES AGAINST THE INFRINGEMENT
OF PLANT BREEDERS' RIGHTS IN JAPAN

Document prepared by experts from Japan

Disclaimer: this document does not represent UPOV policies or guidance

The Annex to this document contains a copy of a presentation "Development of EST-SSR Markers of Lettuce and Variety Identification using EST-SSR Markers" made at the fourteenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in particular (BMT).

Hiroshi GOTO, PVP G-men (Plant Variety Protection Adviser), National Center for Seeds and Seedlings (NCSS), Japan

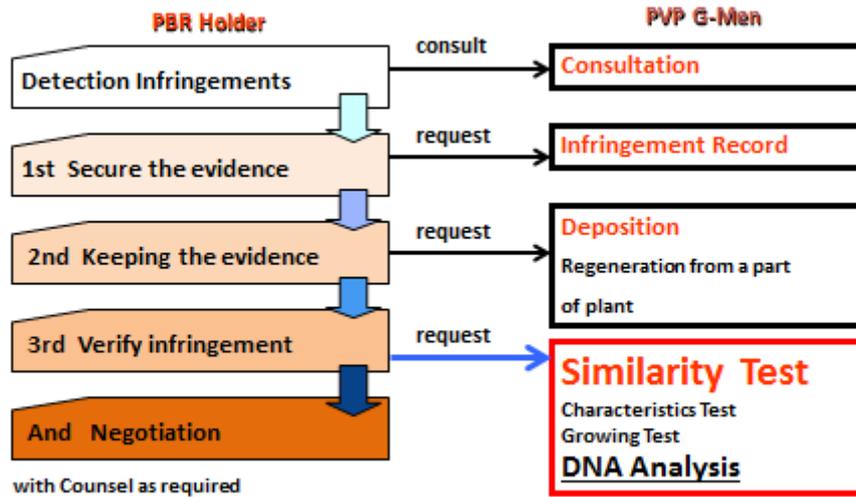
[Annex follows]



CONTENT

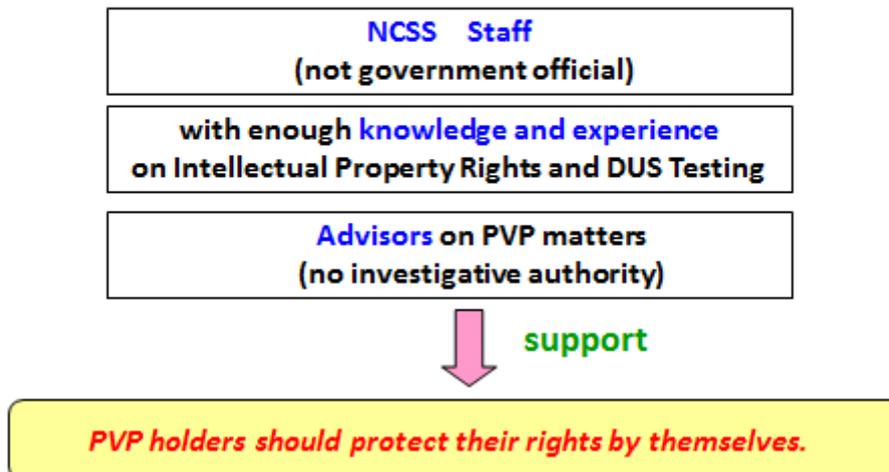
1. Introduction
2. Similarity test by DNA analysis
3. Validation of DNA variety identification techniques
4. Preservation of specimens of protected varieties
5. Conclusion

1. Introduction

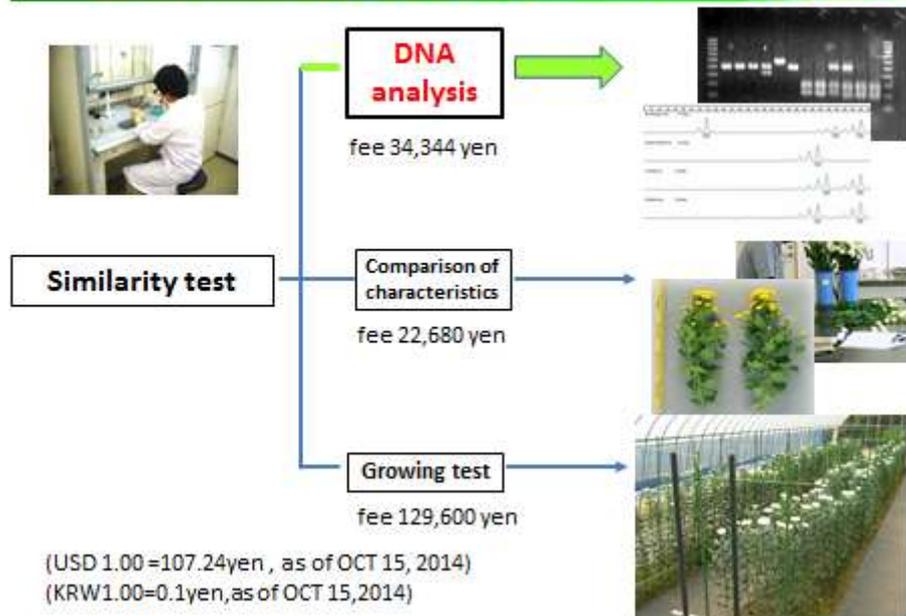


1. Introduction

What is "PVP G-Men" ?



2. Similarity test by DNA analysis



2. Similarity test by DNA analysis

Practical use of Molecular techniques in NCSS

Species	Method
Strawberry (<i>Fragaria x ananassa</i>)	CAPS
Kidney bean (<i>Phaseolus vulgaris</i>)	RAPD-STS
Adzuki bean (<i>Vigna angularis</i>)	RAPD-STS SSR
Igusa Rush (<i>Juncus effusus</i>)	SSR
Tea tree (<i>Camellia sinensis</i>)	CAPS
Sweet cherry (<i>Prunus avium</i>)	SSR
Japanese Pear (<i>Pyrus serotina varcultu</i>)	SSR

CAPS : Cleaved Amplified Polymorphic Sequence
 SSR : Single Sequence Repeat
 RAPD : Random Amplified Polymorphic DNA
 STS : Sequence Tagged Site

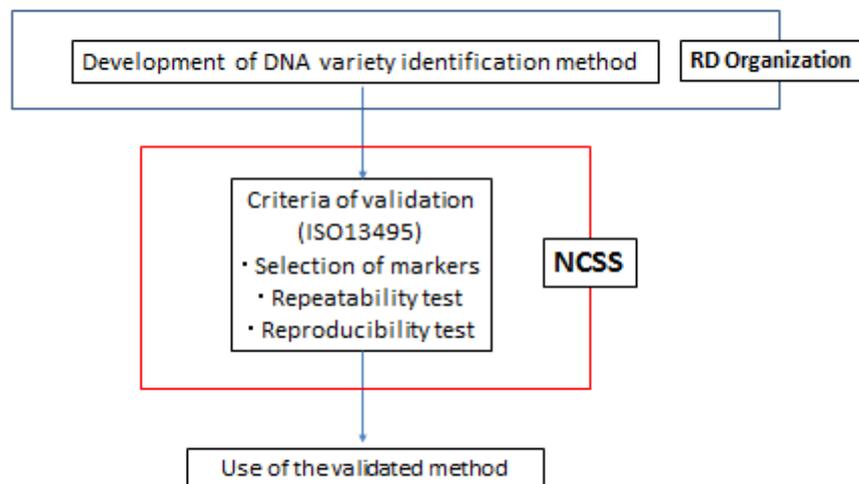
2. Similarity test by DNA analysis

Requirements for DNA analysis method

- DNA variety identification technique is developed and validated
- DNA profiles of varieties put on the market in Japan have been examined, and a variety is clearly distinguishable from any other varieties by using the DNA profiles.

These conditions are met method is SSR

3. Validation of DNA variety identification techniques



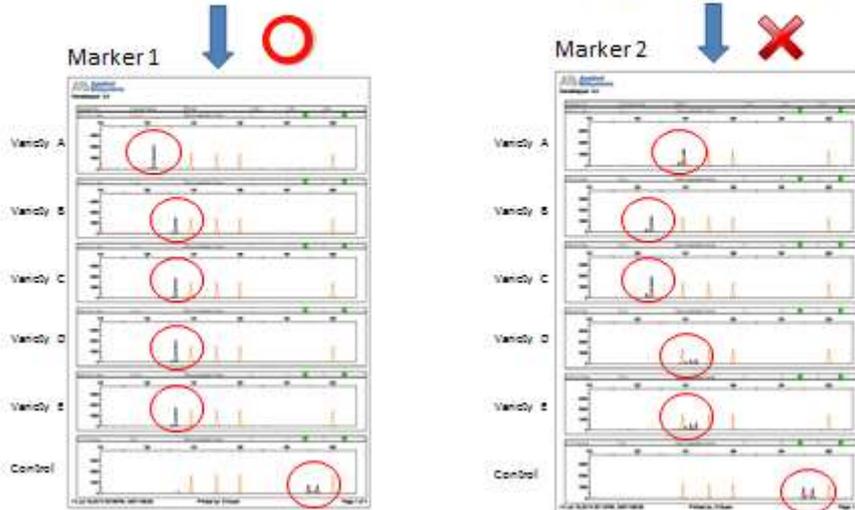
3. Validation of DNA variety identification techniques

○ Fragment evaluation method (Some description of the evaluation method)

Selection of marker

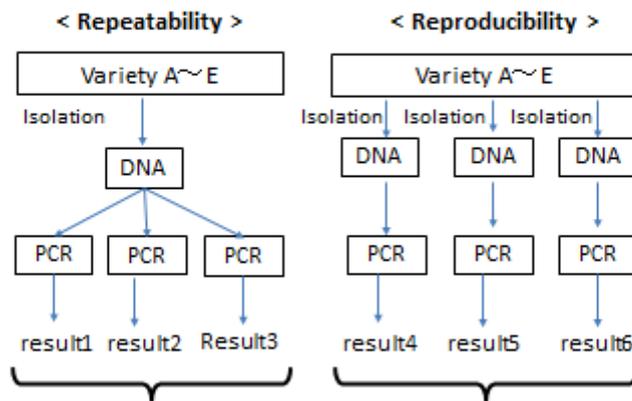
Few or no stutter bands detected

Stutter bands detected.



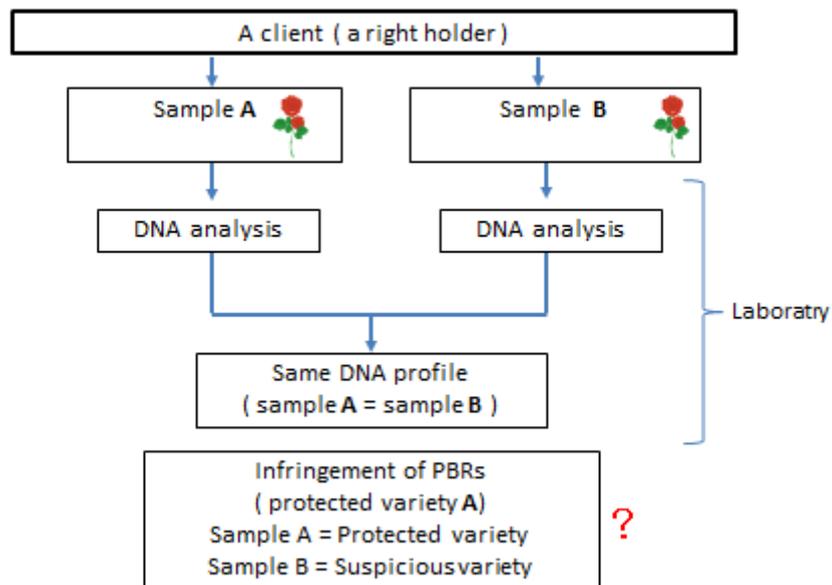
3. Validation of DNA variety identification techniques

NCSS has conducted method validation (intralaboratory evaluation) of the molecular technique according to ISO13495.



The marker is considered reliable (validated).

4. Preservation of specimens of protected varieties



4. Preservation of specimens of protected varieties

Same DNA profile(Sample A=Sample B)

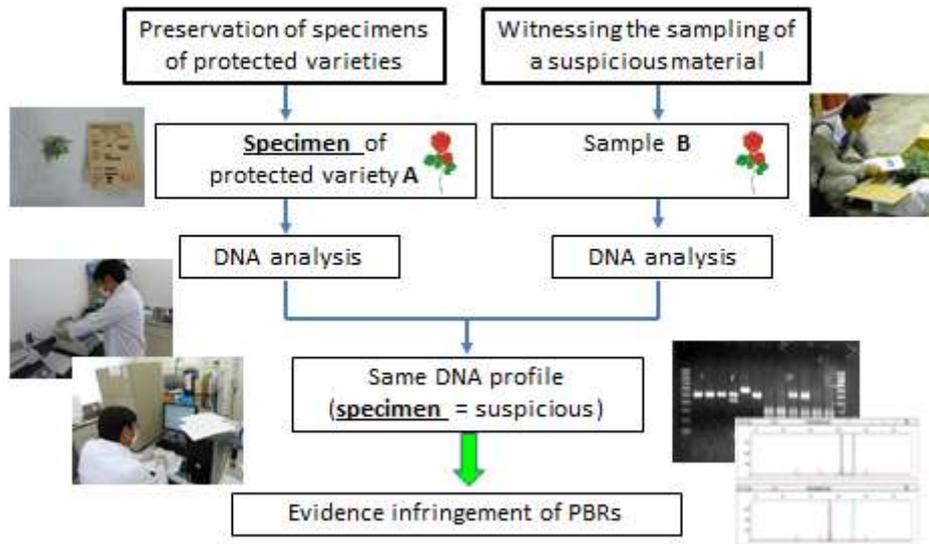
Problem

Even if the DNA profile of samples A matches the DNA profile of sample B, it does not prove infringement of PBRs.

Reason

There is no evidence to confirm that the sample A was from the protected variety. There is no relation between the sample B and infringer.

4. Preservation of specimens of protected varieties



4. Preservation of specimens of protected varieties

Solution

To preserve specimens of protected varieties (freeze-dried leaves); and



① Leaves entered in an envelope

② Freezer -80°C

③ Vacuum freeze dryer

④ Dryness shelf

To witness the sampling of a suspicious material by a client



5. Conclusion

NCSS started the similarity tests service in 2004 and has carried out a total of 170 cases of the tests since then. Out of the 170 cases, 110 cases involved a similarity test using DNA variety identification technique.

Number of similarity tests for DNA analysis

Plant name	No. of similarity test
Igusa Rush	105
Straw berry	4
Sweet Cherry	1

Total	110

(as of April,01,2014)

5. Conclusion

NCSS also started the preservation of specimens of protected varieties since 2008 and has preserved about 3,000 specimens by now.

Plant name	No. of varieties
Rose	414
Chrysanthemum	375
Carnation	182
Strawberry	155
Calibrachoa	85
Citrus	80
Others	1,809

Total	3,000

(as of April,01,2014)



Thank you for your
attention!



National Center for Seeds and Seedlings
<http://www.ncss.go.jp/>

[End of annex and of document]