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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**

Twelfth Session
Ottawa, Canada, May 11 to 13, 2010

ADDENDUM

PROJECT FOR PRESERVING SPECIMENS AND DNA OF
PROTECTED VARIETIES IN JAPAN

Document prepared by experts from Japan

NCSS **The Preservation of specimen and DNA from protected varieties**

登録品種の標本・DNA保存事業のご案内

既に登録品種を保有している品種に限り登録し得ます

新たに取得する品種は登録できません

登録品種の標本・DNA保存事業を活用しましょう

- DNAによる品種識別を実施する際にご利用できます
- 登録品種を証明する資料としてご利用できます
- 新たなDNA品種識別技術開発にも利用されます

※登録された標本・DNAは上記内容にのみ利用されます

登録方法

標本・DNAを採取する

登録申請書・DNA

登録完了

登録完了後

Specimen

DNA

SSR

SNP

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..... AATCAAGGA A CTAAGCTCAG.....
..... AATCAAGGA G CTAAGCTCAG.....
..... AATCAAGGA G CTAAGCTCAG.....
..... AATCAAGGA G CTAAGCTCAG.....
    
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Tetsuya Kimura, Takeshi Sugisawa and Masato Taira
Ottawa, May 11-13, 2010

NCSS **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.

Consultations and advises for infringement cases
(150 cases 2005/4/1-2009/9/30)

- Strengthening of breeder's rights by the revision of the seeds and seedlings law during the period from 2003-2007.


Transition of the number of registrations under breeder's rights continuation

Year	Registrations
83	100
84	150
85	200
86	300
87	400
88	500
89	600
90	700
91	800
92	900
93	1000
94	1100
95	1200
96	1300
97	1400
98	1500
99	1600
00	1700
01	1800
02	1900
03	2000
04	2100
05	2200
06	2300
07	2400
08	2500
09	2600
10	2700
11	2800
12	2900
13	3000
14	3100
15	3200
16	3300
17	3400
18	3500

The total number : 15,530

Transition of the number of registrations under breeder's rights continuation











Crop Type	Percentage
herbaceous ornamental plants	33%
ornamental trees	12%
vegetables	16%
fruit trees	18%
industrial crops	8%
food crops	7%
mushrooms	6%

 **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

Crops (processed foods)	DNA analysis
strawberry 	CAPS 
kidney bean 	RAPD-STS
azuki bean (azuki bean jam) 	RAPD-STS, SSR (SSR)
rice (boiled rice) 	RAPD-STS (SSR)
rush 	SSR 
tea 	CAPS
sweet cherry 	SSR
Japanese pear 	SSR

(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,

Infringement (starburst icon)

PBR holder must provide

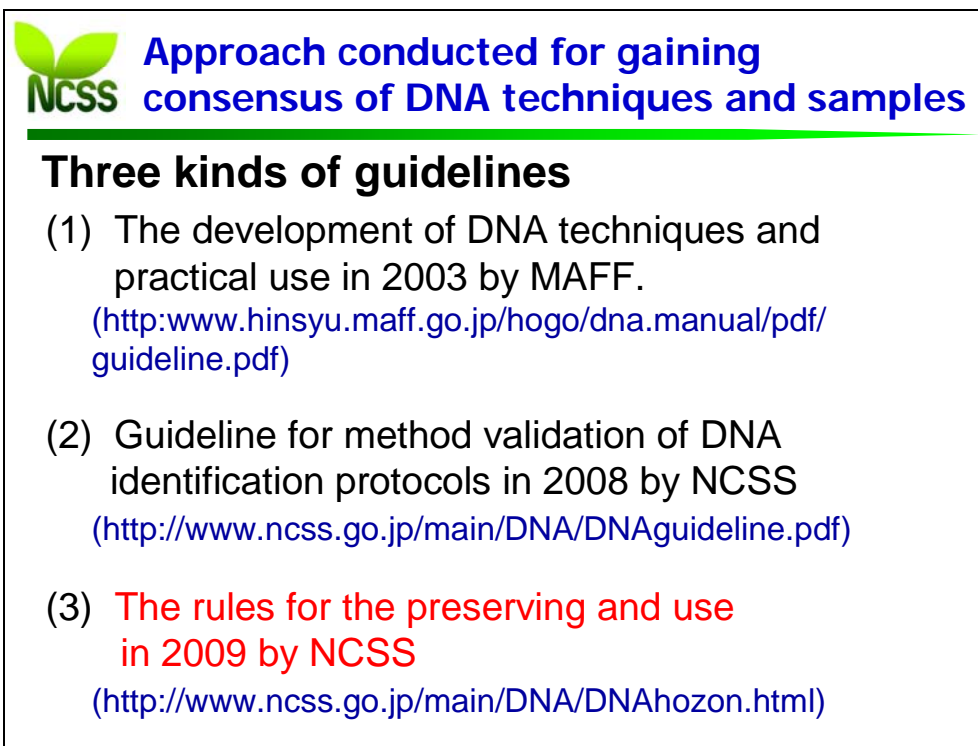
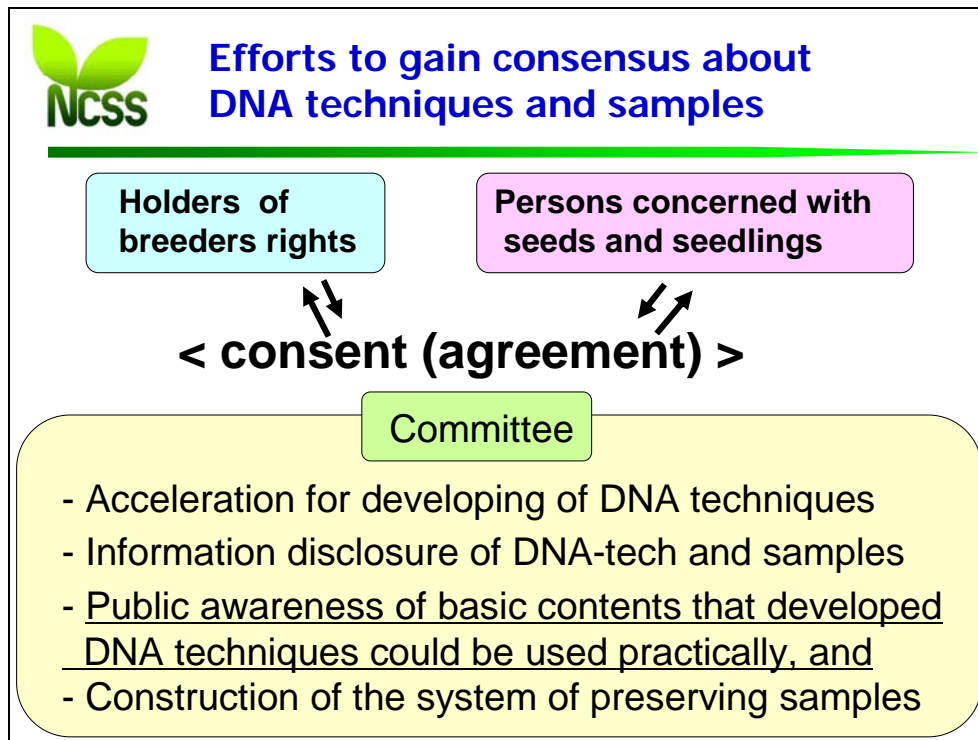
○  →  X


Similarity test

↔ Suspected variety  ↔ Protected variety 

The maintenance and regeneration of original plants were entrusted to PBRs till now.

Characteristics of protected varieties must be maintained for a registration period.



 **Preservation of specimens and DNAs**


Plants of seed propagation
Mushrooms

More than 90% of granted varieties.



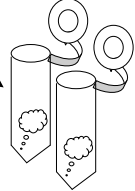
plants of vegetative propagation

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

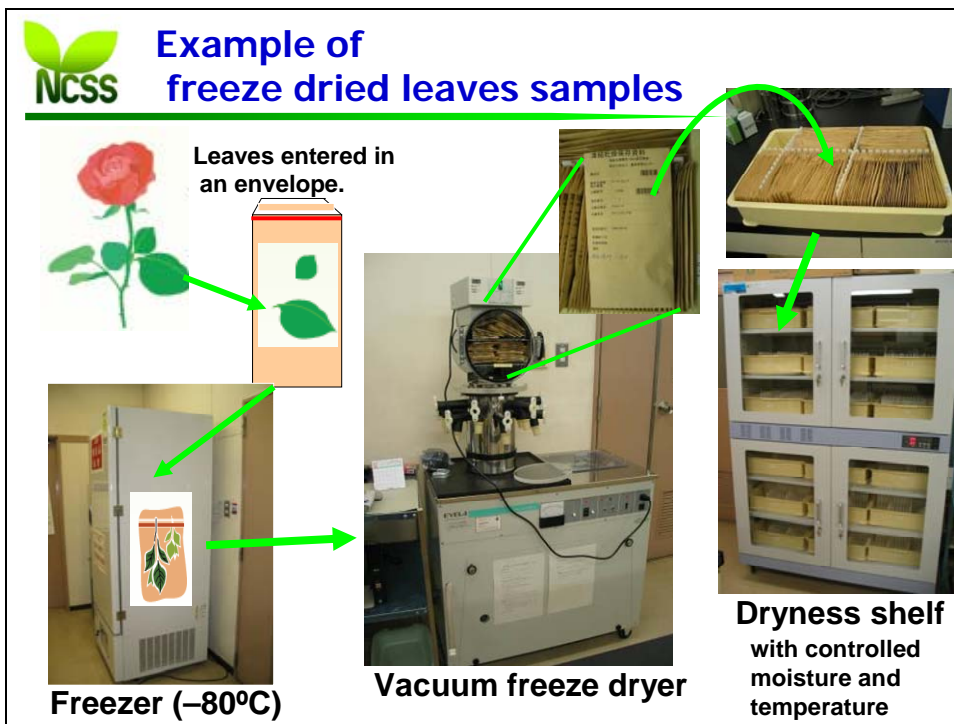
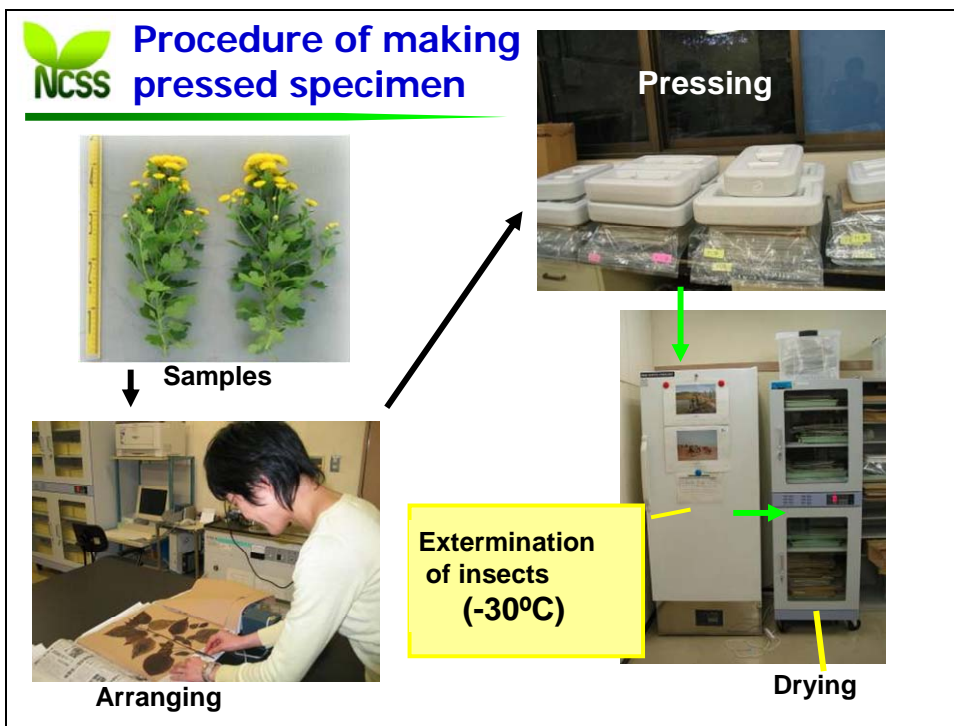
Plants of vegetative propagation are preserved by only the person of PBRs

 **How to preserve**

Three types of samples

- **Specimen** $\xrightarrow{\text{Full bloom stage}}$  An example of pressed specimen
- **Freeze-dried leaves** $\xrightarrow{\text{Leaves of granted varieties are collected and submitted by PBR holders}}$  leaves
- **DNA (-80°C)** $\xrightarrow{\text{Genomic-tip20/G method (QIAGEN)}}$  Genomic DNA

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



NCSS **DNA preservation**

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

Freezer (-80 °C)

Example of DNA tube

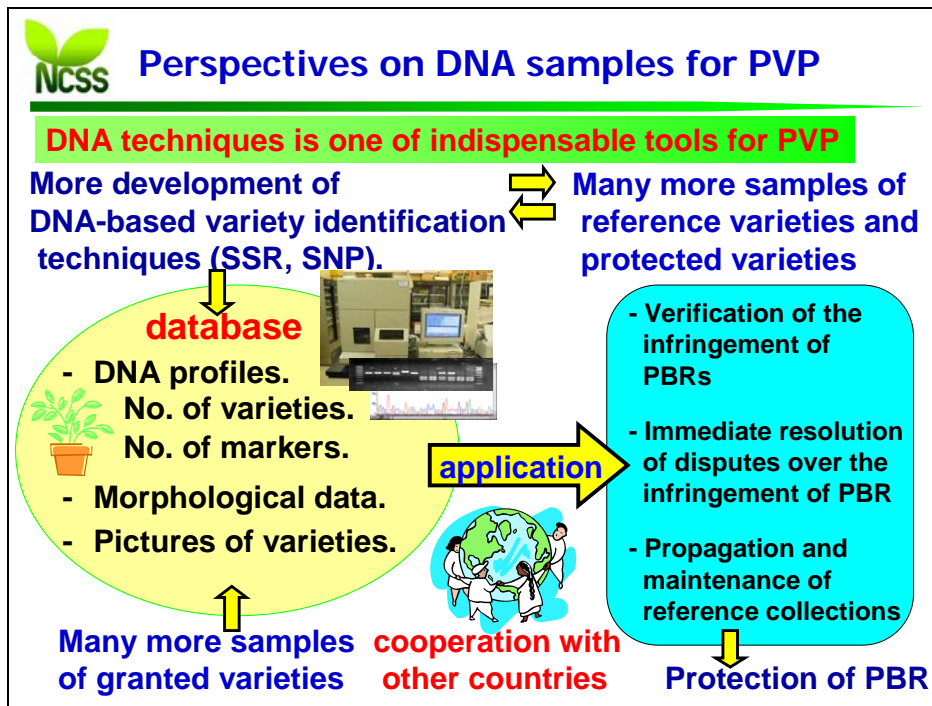
purification

Function Autocalibrate Autoelute Print
Sensitivity: None
Wavelength (nm)

NCSS **No. of varieties preserved in NCSS**

Plant name	No. of varieties	2010/3/10
Chrysanthemum	219	
rose	127	
carnation	115	
strawberry	95	
Citrus	53	
Calibrachoa	52	
petunia	43	
peach	34	
others	834	
total	1,572	

Samples are managed with a computer



NCSS *Acknowledgements*

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

- Tsukuba university
- National Institute of Floricultural Science
- 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

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Division and DUS Test Division