Role of

DUS test

2. Examination of DUS

Workflow of the DUS test

Checking Characteristics of Candidate Variety

→

1. Selecting varieties for the trial
(Similar Varieties, Example varieties)

↓

Growing trial

←

2. Observation of Characteristics

←

3. Assessing DUS
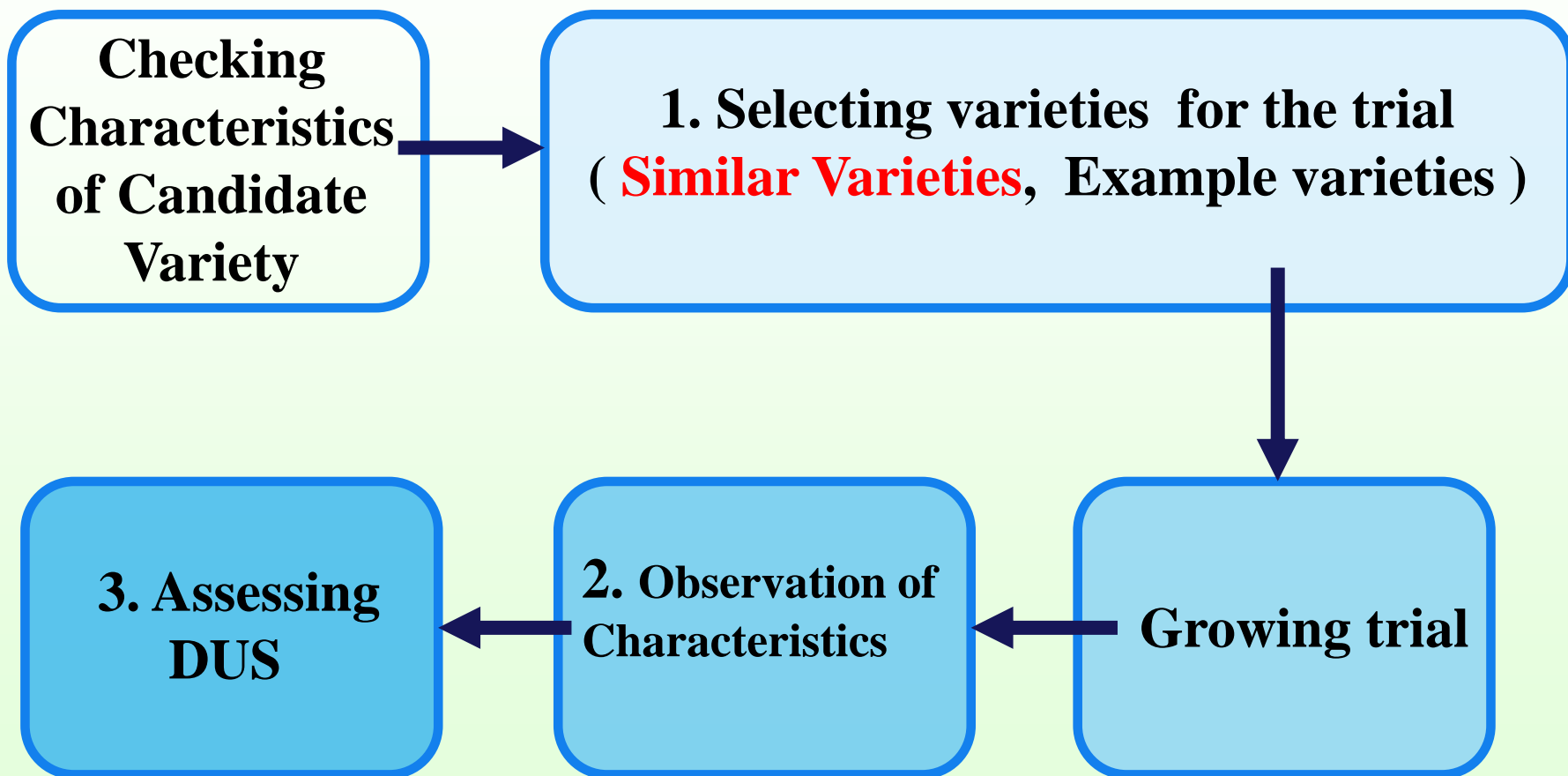
DUS test

DUS test

Candidate variety

Similar varieties

Example varieties

Similar varieties: Varieties very close to the candidate varieties in morphological, physiological characteristics

Example varieties: Varieties to clarify the states of expression of a characteristic, then to assist with preparation of the description

3

Distinctness examination

- **Selection of similar varieties**

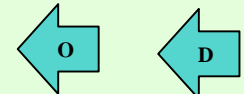- **Clearly distinguishing new variety**

Distinctness

Requirement:

■ The variety shall be deemed to be distinct if it is clearly distinguishable from any other variety whose existence is a matter of common knowledge at the time of the filing of the application.

TG/1/3 5.3.3

■ A variety may be considered to be clearly distinguishable if the difference in characteristics is:

(a) consistent, and

(b) clear.

clearly distinguishable => 1. Consistent
2. Clear

Clearly Distinguishable

from any other varieties **?**

"it is necessary to examine distinctness in relation to all varieties of common knowledge. However.."

VS

Compare   Candidate variety VS Existing varieties 6

Selection of Similar Varieties

Where a candidate variety is sufficiently different from particular group of varieties,

VS

No need to compare the candidate variety with different group of varieties
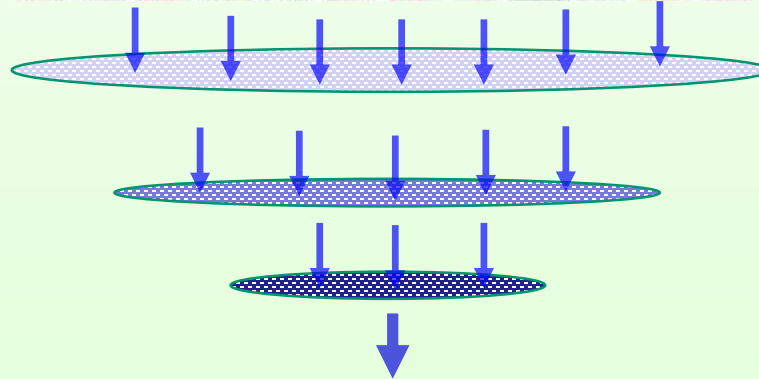
How to select different group of varieties?

Selection of Similar Varieties

Selecting the similar varieties

Candidate varieties

Grouping characteristics

Similar varieties

Grouping characteristics

- **Grouping characteristics: Cymbidium**

    (a) Plant: size (char. 1)

    (b) Inflorescence: number of flowers (char. 20)

    (c) Peduncle: attitude (char. 24)

    (d) Flower: general impression of petals and sepals (char. 28)

    (e) Flower: length (char. 29)

    (f) Flower: width (char. 30)

    (g) Flowering time (char. 100)

    (h) Flower: predominant color (Technical Questionnaire 5.8)

Selection of Similar Varieties

VS

No need to compare candidate variety with different group of varieties

How to select different group of varieties?

Grouping characteristics

■ **Grouping characteristics: Tomato**

(a) Plant: growth type (characteristic 2: QL)

(b) Leaf: type of blade (characteristic 10: QL)

(c) Peduncle: abscission layer (characteristic 19:QL)

(d) Fruit: green shoulder (before maturity) (characteristic 21:QL)

(e) Fruit: size (characteristic 26:QN)

(f) Fruit: shape in longitudinal section (characteristic 28:PQ)

(g) Fruit: number of locules (characteristic 36:QN)

(h) Fruit: color (at maturity) (characteristic 3:PQ)

Selection of Similar Varieties

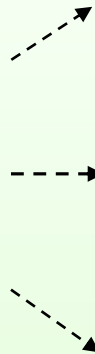*No need to compare the candidate variety with different group of varieties*

VS

Selection of Similar Varieties
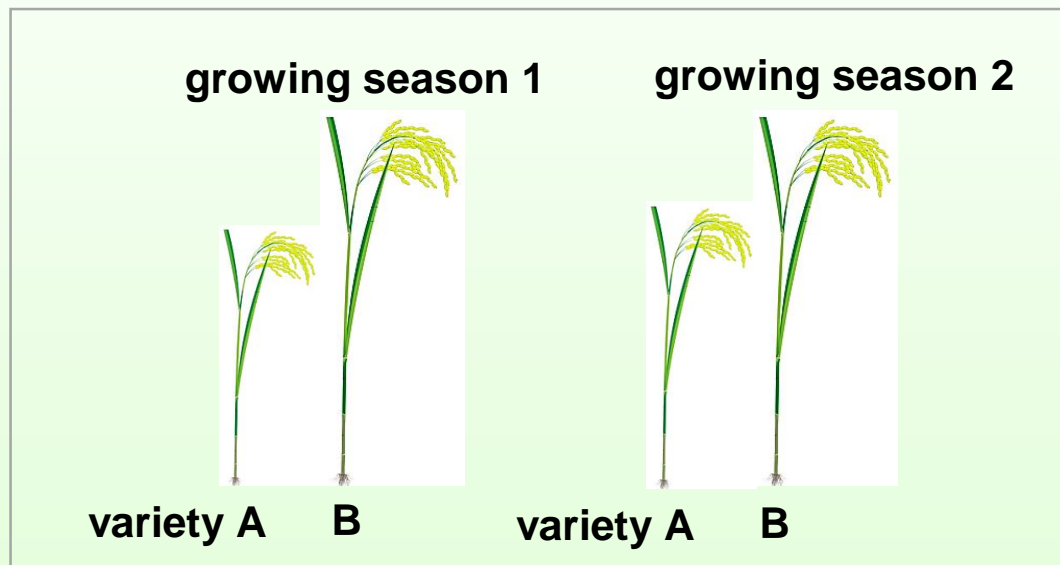

Candidate varieties

≠

≠

≠

Similar varieties

the candidate variety is considered to be distinguished to all existing varieties

Distinctness

1.Consistent difference:

To ensure sufficient consistent is to examine the characteristics in at two independent growing cycles.

Differences have to occur in two growing cycles

Distinctness

2.Clear differences: TG/1/3： 5.3.3.2

Determining whether a difference between two varieties is clear **depends on the type of expression of the characteristics**.

QL: Qualitative

QN: Quantitative

PQ: Pseudo-Qualitative

QL

Distinctness examination

Distinctness

QL **characteristics:**

TG/1/3： 5.3.3.2.1

Requires:

■ the difference between two varieties may be considered clear if one or more characteristics have expressions that **fall into two different states in the Test Guidelines**

Different "states" can be considered to be Distinct

17

Distinctness

Different "states" can be Distinct

Fruit：green shoulder (before maturity)

Absent 1   Present 9

Stem: anthocyanin coloration of nodes

Absent 1   Present 9

QN

Distinctness examination

Distinctness

QN **characteristics:**

TG/1/3： 5.3.3.2.2

■ For QN, **a difference of two Notes often represents a clear difference**, but that is not an absolute standard for assessment of distinctness. Depending on factors, such as the testing place, the year, environmental variation or range of expression in the variety collection, a clear difference may be more or less than two Notes. Guidance is provided in document TGP/9, 'Examining Distinctness'."
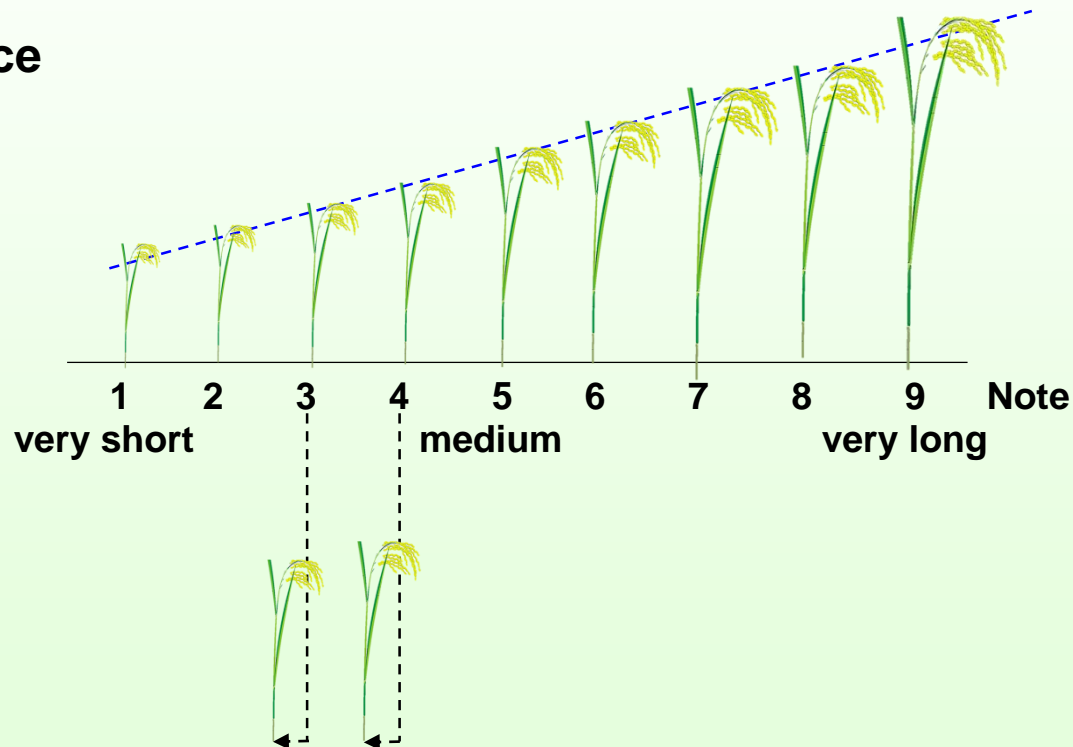
✓ **"Two Notes" rule**

20

Distinctness

QN **characteristics:**

(stem: Length)

clear difference

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Note |

very short   **medium**   **very long**

Note 3: 4 → may NOT be clear difference

21

Distinctness

QN characteristics:

(stem: Length)

clear difference

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Note |

very short   **medium**   **very long**

Note 3: 5 → may be clear difference

22
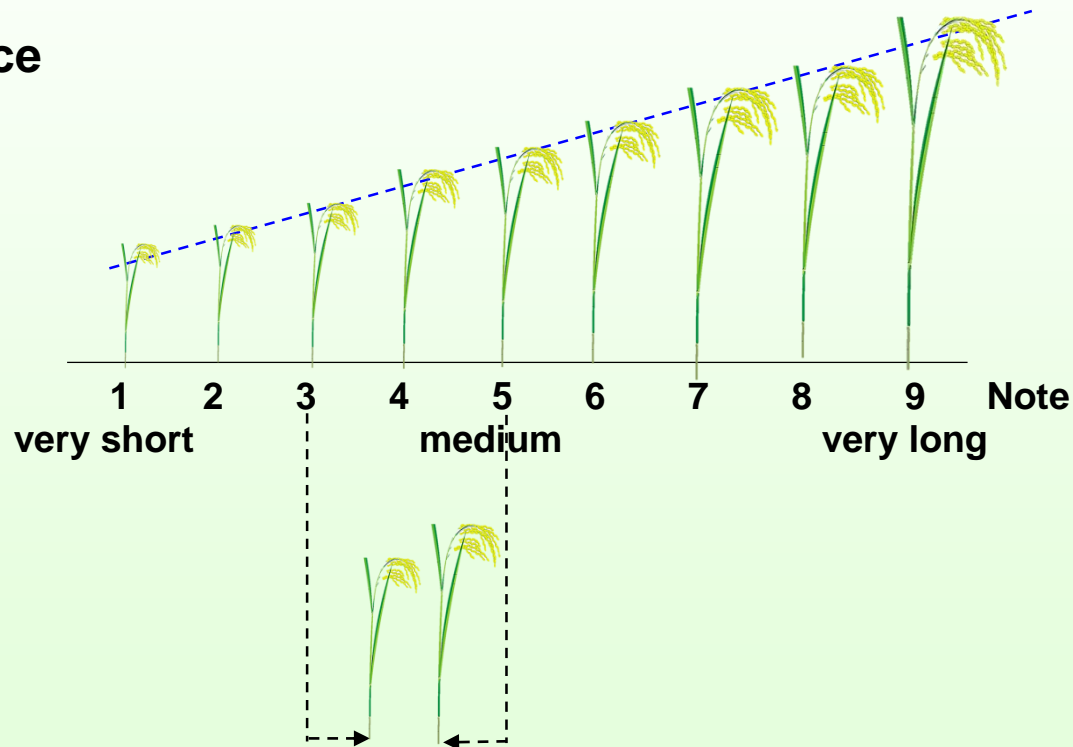
Distinctness

"a difference of two Notes often represents a clear difference"

"Two Note" rule

QN chars. (Length, Height） | ● Variety **A** | ● Variety **B**

	1	2	3	4	5	6	7	8	9		
case 1			●		●					Note3 : Note5	**Different**
case 2			●	●						3 : 5	**Different**
case 3				●	●					3 : 5	?
case 4					● ●					4 : 5	?
case 5				●	●					4 : 5	?
case 6				●	●					4 : 5	?
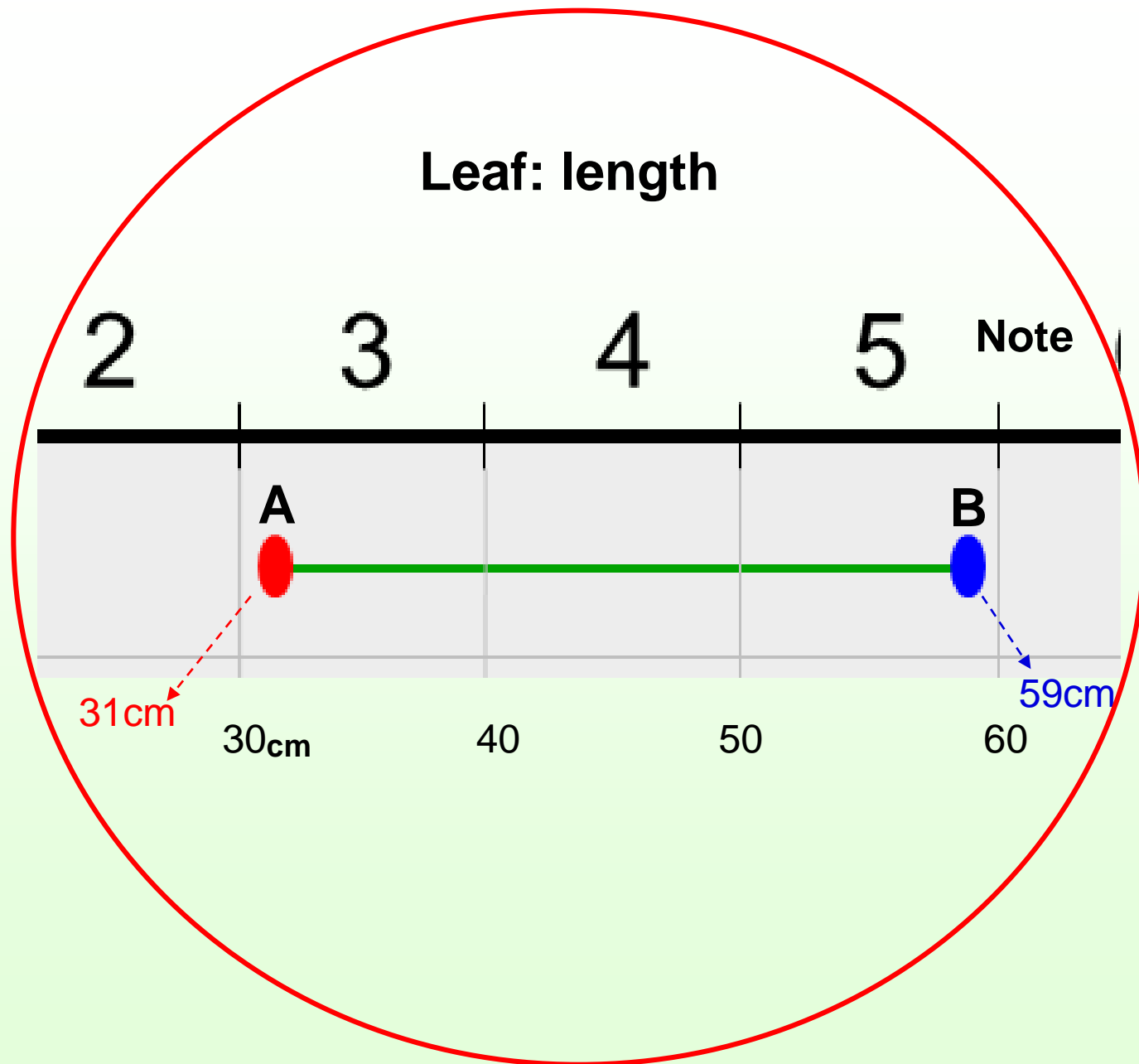
Distinctness

Clear difference

QN: Stem: Length

	2	3	4	5	6	Note
case 3			●━━━━━━●			3:5
case 6			●━━━━━━━━━●			4:5
	30	40	50		60cm	
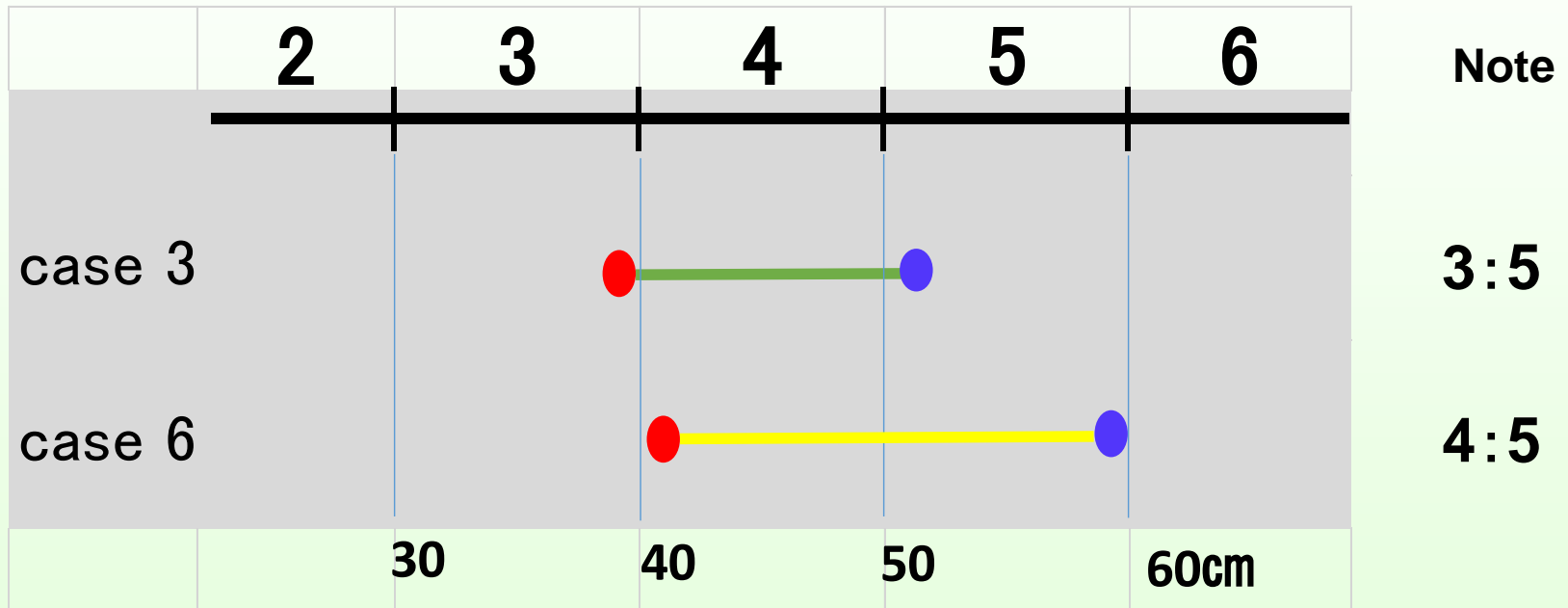
case 3 < case 6

"Two Notes" rule means at least <u>One note difference</u>

Distinctness

■ **Verity: Combination of expression of characteristics**

char No.	8	9	15	16	24	36	39	41
characterisitics	Tassel: time of anthesis	Tassel: anthocyanin coloration at base of glume	Ear: time of silk emergence	Ear: anthocyanin coloration of silk	Plant: length	Ear: type of grain	Ear: color of dorsal side of grain	Ear: anthocyanin coloration of glumes of cob
Candidate variety	5	5	5	5	5	1	4	1
existing variety 1	5	5	5	5	5	3	4	1
variety 2	5	3	5	5	5	1	4	1
variety 3	5	5	3	5	5	1	4	1
variety 4	5	5	5	5	7	1	4	1

New verity: new combination of expression of characteristics

26

PQ

Distinctness examination

Distinctness

PQ **characteristics:**

TG/1/3： 5.3.3.2.3

- ■ **A different state in the Test Guidelines may not be sufficient to establish distinctness** (see also section 5.5.2.3). However, in certain circumstances, varieties described by the same state of expression may be clearly distinguishable.

  - ✓ difficult to define a general rule on the difference in Notes to establish Distinctness

  - ✓ need to compare the state of expression directly side by side in the field

Distinctness

PQ: clear difference
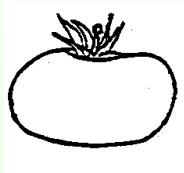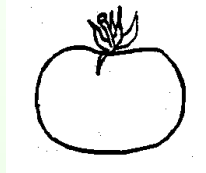
1. flattened 2. oblate 3. circular 4. blong 5. cylindric 6. elliptic
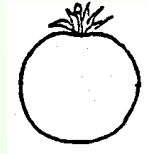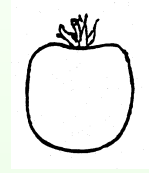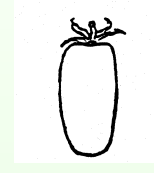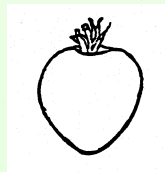
7. cordate 8. ovate 9. obovate 10. pyriform 11. obcordate
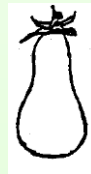
Distinctness

Clear difference

Distinctness

	characteristics	Assessment
QL	- **discontinuous states** - **absent / present**	**different states**
QN	- **continuous states** - **length, width**	**two notes rule**
PQ	- **more than one dimension** - **shape, color**	**A different state in the TGs may not be sufficient**

Uniformity

■ **Assessment of Distinctness**

Method of propagation of the variety	QL	PQ	QN
Vegetatively propagated, self-pollinated	Notes(VG)	Notes(VG) Side-by-side(VG)	Notes(VG) Side-by-side(VG) Statistics(VS)
Cross-pollinated	Notes(VG) Statistics(VS*)	Notes(VG) Side-by-side(VG) Statistics(VS*)	Statistics(VS) Side-by-side(VG) Notes(VG)
Hybrid	Notes(VG) Statistics(VS*)	Notes(VG) Side-by-side(VG) Statistics(VS)	according to the type of hybrid

*   Records of individual plants only necessary if segregation is to be recorded.
•   The most common approach are listed first.

32

U
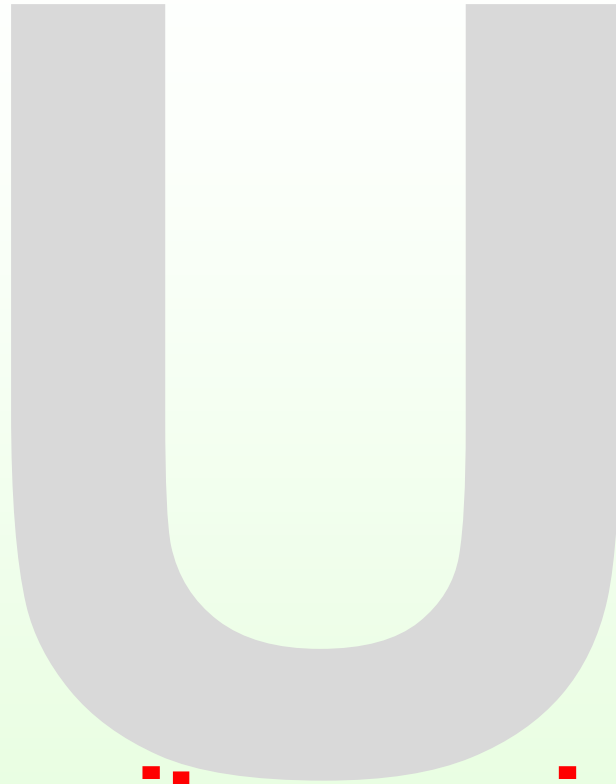
Uniformity examination

- **Features of propagation of the variety**

Uniformity

Requirement: Article 8; 91 Act of the UPOV

- The variety shall be deemed to be uniform if, <u>subject to the variation that may be expected from the</u> particular features of its propagation, it is sufficiently uniform in its relevant characteristics.

✓ *level of uniformity required for the variety will be different*

34

Uniformity

■ **Features of propagation of the variety**

Features of propagation	Genetic variation	How to assess U
•Vegetatively propagated	Lower	?
•Truly Self-pollinated		?
•Mainly Self-pollinated		?
•Cross-pollinated	Higher	?
•Hybrids		?

Uniformity

■ **Assessment of Uniformity**

Method of propagation of the variety	QL	PQ	QN
Vegetatively propagated	Off-types	Off-types	Off-types (Visual observation) Standard deviations (measurement)
Self-pollinated	Off-types	Off-types	Off-types (Visual observation) Standard deviations (measurement)
Cross-pollinated	Off-types	Off-types	Standard deviations
Single hybrid (inbred parent lines)	Off-types	Off-types	Off-types (Visual observation) Standard deviations (measurement)
Other hybrid	according to the type of hybrid		
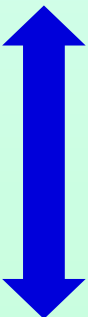
- The most common approaches are listed first.

Uniformity

■ **Methods for Examining Uniformity**

    1. **Off-types approach**
    2. **Standard deviation approach**

Features of propagation	Genetic variation	U assessment
•Vegetatively propagated	**Lower**	Off-types
•Truly Self-pollinated		Off-types
•Mainly Self-pollinated		Off-types
•Cross-pollinated	**Higher**	standard deviation
•Hybrids		depends on type of hybrid

Uniformity

1. Off-types approach

Where all the plants of a variety are very similar,
for vegetatively propagate and self-pollinated varieties,
Uniformity is assessed by the number of off-types

How many off-types should we accept?

Uniformity

How many off-types should we accept?

According to the **size of the sample** examined, statistical tables give the maximum **number of off-types** tolerated in that given samples

e.g.:　　　　　*population standard = 1% and*

acceptance probability = 95%

Sample size	Number of off-types allowed
1-5	0
6-35	1
36-82	2
83-137	3
138-198	4
199-262	5

Uniformity

How many off-types should we accept?

- **Population standard**
  (Acceptable Number of off-types)
  - ✓ **Percentage of off types to be accepted** if all individuals of the variety could be examined

- **Acceptance probability**
  - ✓ Probability of correctly accepting that a variety is uniform

Uniformity

species and genera	Assessment of uniformity
soya bean	a population standard (P.S.) of 0.5% with an acceptance probability(A.P) of at least 95% should be applied. In the case of a sample size of 300 plants, the maximum number of off-types allowed would be 4.
tomato	P.S. of 1% and A.P. of at least 95% should be applied. In the case of a sample size of 20 plants, 1 off-type is allowed.
chrysanthemum	P.S. of 1% and A.P. of at least 95 % should be applied. In the case of a sample size of 20 plants, 1 off-type is allowed.
apple	P.S. of 1% and A.P. of at least 95% should be applied. In the case of a sample size of 5 plants, no off-types are allowed. In the case of a sample size of 10 plants, 1 off-type is allowed.
banana	P.S. of 1% and A.P. of at least 95% should be applied. In the case of a sample size of 15 plants, 1 off-type is allowed.
tulip	P.S. of 1% and A.P. of at least 95 % should be applied. In the case of a sample size of 25 plants, 1 off-type is allowed.
sugarcane	P.S. of 1% and A.P. of at least 95% should be applied. In the case of a sample size of 6 culms 1 off-type is allowed. In the case of a sample size of 24 culms, 1 off-type is allowed.

Uniformity

■ **Acceptance probability**

case:

       sample size ; 100 plants
       acceptance probability; 95%

Population standard							
Acceptance Probability 95 %	10%	5%	5%	2%	1%	0.5%	0.1%
Allowed number of off-types	15	9	6	5	3	2	1

PS, AP in each UPOV TGs

population standard	Acceptance probability	sample size	Number of off types	species
0.1	95	1500	4	Rice
0.1	95	2000	5	Durum wheat
1	95	5	0	Almond, Blueberry, Persimmon, Avocado, coffee, fig,Dragon fruit, Mango
1	95	6	1	Nerium, BirdCherry, Buddleja, Papaya
1	95	7	1	Eucalyptus,Rubber
1	95	8	1	Alstromeria, Hydrangea, Clematis, Rose of Sharon, Canna, Hebe
1	95	9	1	Phalaenopsis, Oncidium
1	95	10	1	Bougainvillea, Camellia, Pineapple, Dendrobium, TeaTree, Brachyscome, Poinsetia
1	95	12	1	Dahlia
1	95	15	1	ZonalPelargonium, Banana, Lobelia, Osteospermum, Sutera
1	95	20	1	Yam, Peppermint, Pumpkin, Tomato, Lily, Melon, Gladiolus, Chrysanthemum
1	95	24	1	sugarcane
1	95	25	1	tulip
1	95	40	2	bitter gourd, asparagus, Brussels sprout,cucumber, Petunia, Antirrhinum,Onion
1	95	50	2	Amaranth, Sweet potato, Sesame
1	95	60	2	cornsalad, chinese Cabbage, broccoli, Calabres sprouting, chimes Chive, Shiitake
1	95	90	3	Oyster Mushroom
1	95	100	3	Chick Pea, Lentil
2	95	20	2	Elatior Begonia, Kalanchoe,Chili,Watermelon,
2	95	200	7	Beetroot, Carrot,Leek, Radish, Black Radish
3	95	40	3	Maize
5	95	40	4	Artichoke, Cardoon
Hybrids:2 inbred:2	Hybrids:95 inbred:95	Hybrids:100 inbred:200,30	Hybrids:5 inbred:7,2	Parsnip
Hybrids:2 inbred:3	Hybrids:95 inbred:95	Hybrids:100 inbred:100	Hybrids:5 inbred:6	Spinach,
inbred:1 (s)cross:3	inbred:95 (s)cross:95	inbred:60 (s)cross:60	inbred:2 (s) cross:4	Cauliflower

Uniformity

Off type

Off type

Off type
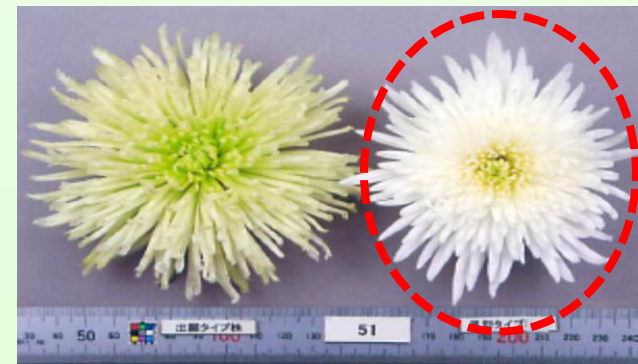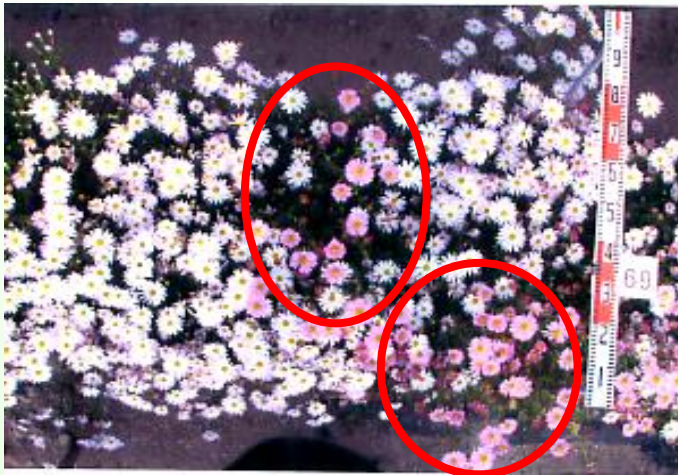
Uniformity

Uniformity

Off-type

TG/1/3
 6.4.1.1 Determination of Off-Types by Visual Assessment

■ A plant is to be considered an off-type if it can be ***clearly distinguished from the variety*** in the expression of any characteristic of the whole or part of the plant that is used in the testing of distinctness, taking into consideration the particular features of its propagation.

clearly distinguished from the variety **= same criteria as for Distinctness**

Stability examination

Stability

Requirement:

- The variety shall be deemed to be stable if its relevant characteristics remain unchanged after repeated propagation or, in the case of a particular cycle of propagation, at the end of each such cycle.

- In practice, it is not usual to perform tests of stability that produce results as certain as those of the testing of distinctness and uniformity.

- However, for many types of variety, when a variety has been shown to be uniform, it can also be considered to be stable

Stability

- Where appropriate, or in cases of doubt, stability may be tested, either by growing a further generation, or by testing a new seed or plant stock to ensure that it exhibits the same characteristics as those shown by the previous material supplied.

- Stability needs appropriate maintenance of the variety by the breeder continuously.

relevant characteristics:
The relevant characteristics include at least all characteristics used for the examination of DUS or included in the variety description established at the date of grant of protection of that variety.

49

Summary

DUS test
- Object: to assess whether the variety meets the DUS requirement
- Definition of the variety ---> To generates a description of the candidate variety, using its relevant characteristics (e.g. plant height, leaf shape, time of flowering)
- Assessing DUS

Observation of characteristics
- type of expression — QL,QN,PQ
- Method of observation — V, M
- type of record — G, S

General Introductio to DUS

D Examination
- selection of similar varieties — grouping characteristics
- examining D — type of expression — QN / QL / PQ

U Examination
- level of uniformity requirement for a variety will be different — acceptable number of off-types
  - Vegetatively, Truly self-pollinated varieties
  - Mainly self-pollinated, Inbred lines of Hybrid varieties
  - Cross-pollinated varieties

S Examination
- a variety has been shown to be uniform, it can be considered to be stable

Thank you for your attention

MIZUNO Tadao
tadao.mizuno@gmail.com

Final Goal

■We can carry out DUS test for Tomato

Step

1. *To understand basic principles of examination of DUS based on UPOV system*
2. *To understand deeply how to assess D,U,S for Tomato by practical work*
3. *To understand for making a DUS test report*