How to assess DUS

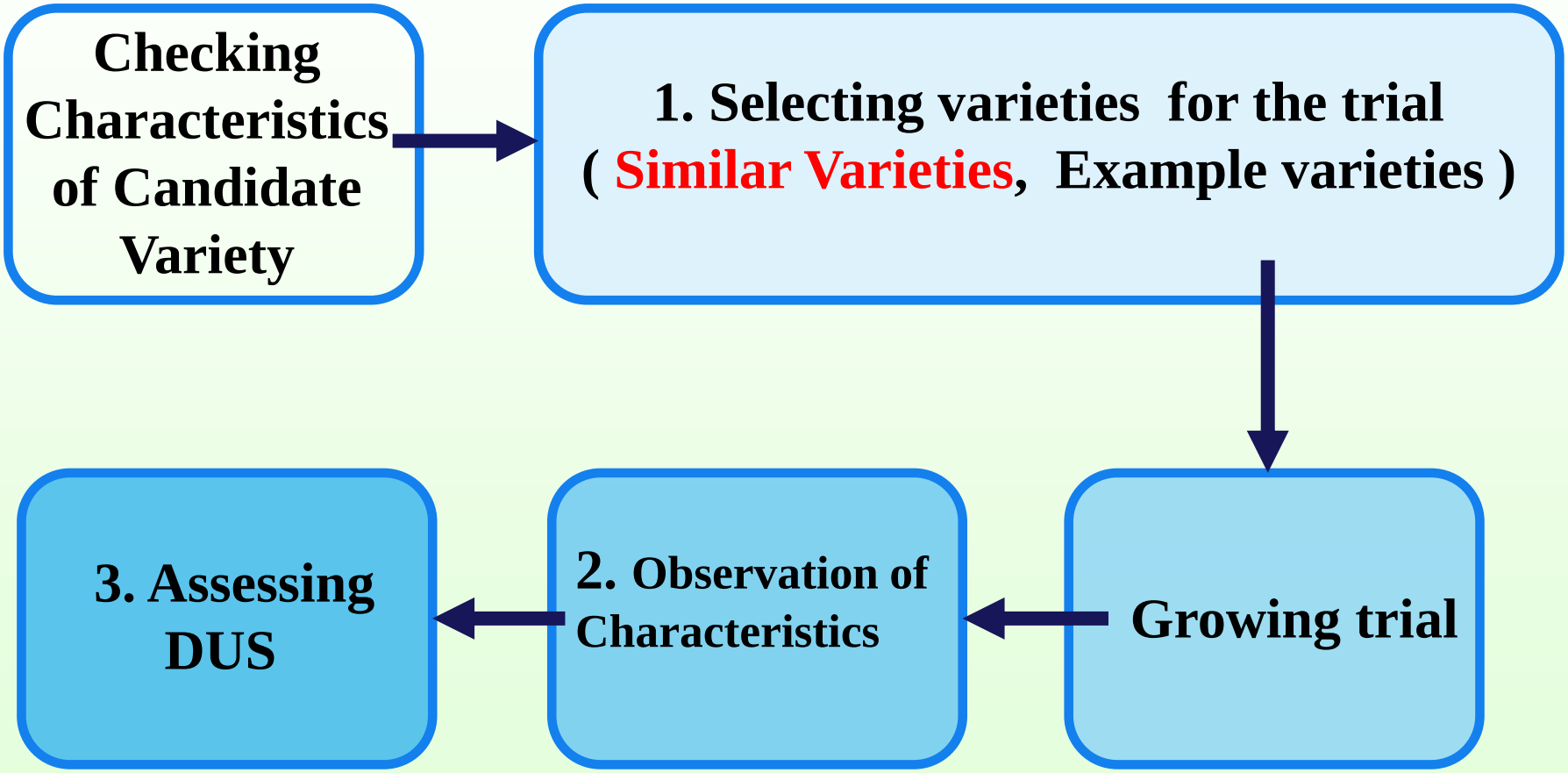
Tadao Mizuno
tadao.mizuno@gmail.com

2018. Jan. 23

Contents

1. Distinctness
   - consistent difference
   - clear difference
2. Uniformity
   - off-type approach
   - standard deviation approach
3. Stability

Workflow of the DUS test



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

Selection of reference similar variety

Clearly Distinguishable

from any other varieties ?

TG/1/3: 5.3.1.1

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

VS

Compare Candidate variety VS Existing varieties

Selection of Similar Varieties

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

VS

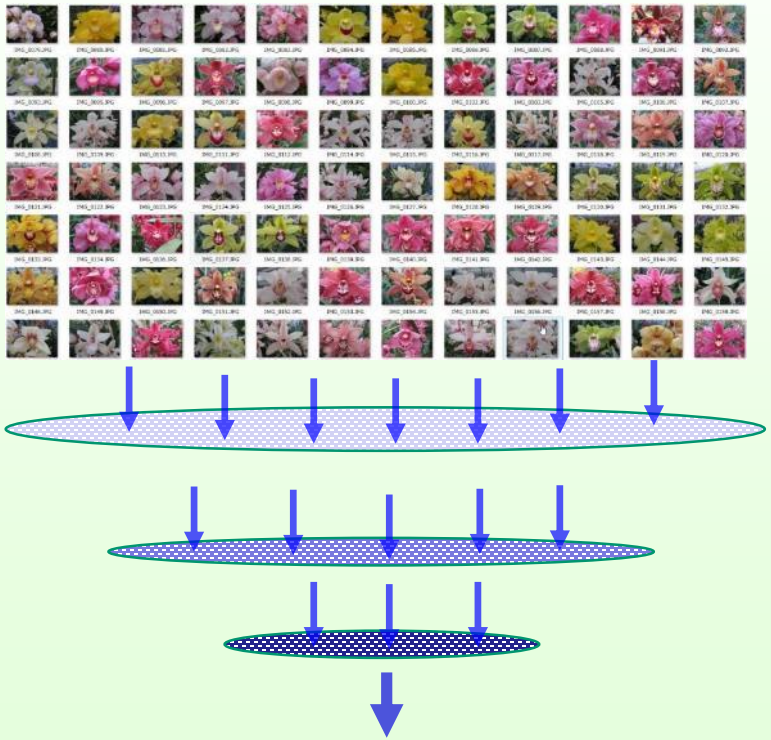
No need to compare the candidate variety with different group of varieties

How to select different group of varieties?

Selection of Similar Varieties

Selecting the similar varieties

Candidate varieties



Grouping characteristics

Similar varieties

Selection of Similar Varieties

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

VS

D

Distinctness examination

- consistent difference
- Clear difference

Distinctness

Requirement:

Article 7; 91 Act of the UPOV

- 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

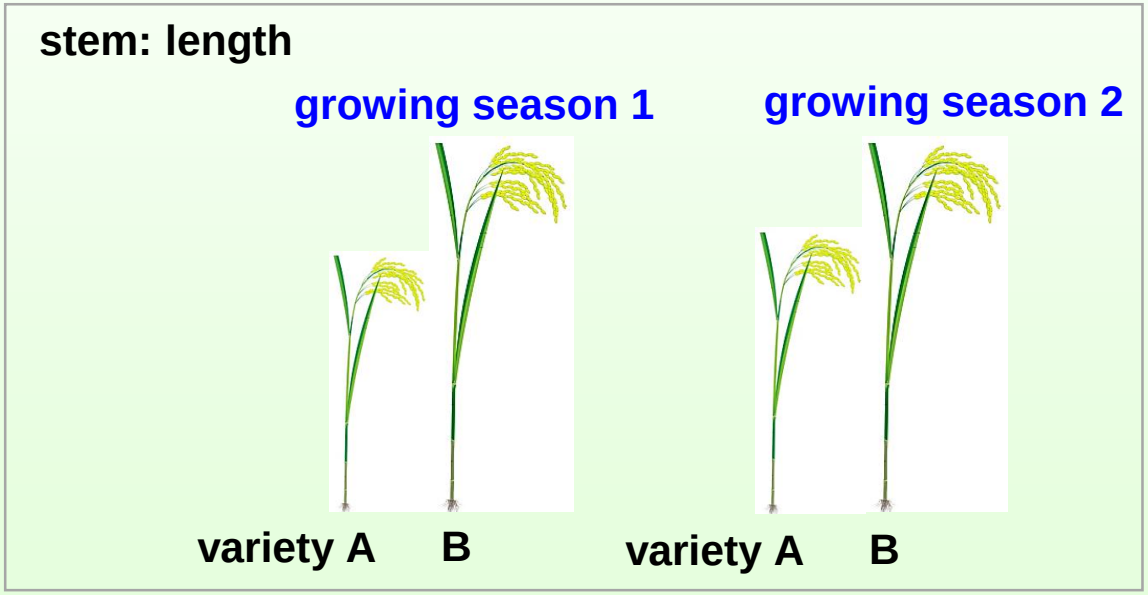
(b) clear.

clearly distinguishable => 1. Consistent
2. Clear

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

1.Consistent difference (cont.):

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

- ✓ If the growing conditions of the crop are controlled, such as in a greenhouse with regulated temperature and light, it may not be necessary to observe two growing cycles.
- ✓ The differences observed between varieties could be so clear that a second growing cycle may not be necessary.
- ✓ The individual Test Guidelines specify whether several independent growing cycles are required to show sufficient consistency

Distinctness

Consistent difference

Test guidelines

- ***3.1 Number of growing cycles (duration of tests)***

species and genera	growing cycle
Rice, maize, soya bean	The minimum duration of tests should normally be two independent growing cycles.
Tomato, cabbage	two independent growing cycles.
Chrysanthemum, Tulip	a single growing cycle
Banana, Mango	two independent growing cycles. It is essential that the plants produce a satisfactory crop of fruit in each of the two growing cycles. In particular, observations should not be made on the first crop of fruit
sugarcane	a single growing cycle

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

Clear difference

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

Distinctness

Clear difference

Different ”states” can be Distinct --> note1 : 9

Fruit：green shoulder (before maturity)

Absent 1　　Present 9

Stem: anthocyanin coloration of nodes

Absent 1　　Present 9

Distinctness

Clear difference

Different ”states” can be Distinct --> note1 : 2

TG/219/1 Perilla

13. (*)	VG	Leaf blade: color of lower side	Note
QL	(a)	greenish	1
		purplish	2

purplish 2

TG/221/1 Antirrhinum

15. (*) (+)		Flower: form	Note
QL	**(c)**	zygomorph	1
		actinomorph	2

http://garden-vision.net

zygomorph 1

https://www.anniesannuals.com

actinomorph 2

PQ

Distinctness examination

Distinctness

Clear difference

PQ characteristics:

TG/1/3： 5.3.3.2.3

- **A different state in the Test Guidelines may not be sufficient to establish distinctness**.

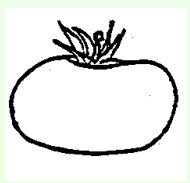
However, in certain circumstances, varieties described by the same state of expression may be clearly distinguishable.

- ✓ 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

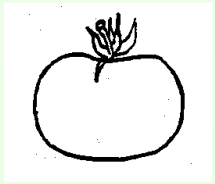
Distinctness

Clear difference

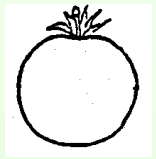
PQ: clear difference



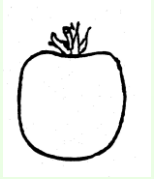
1.flattened



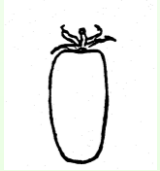
2.oblate



3.circular

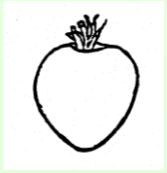


4.oblong



5.cylindric

6.elliptic



7.cordate

8.ovate

9.obovate

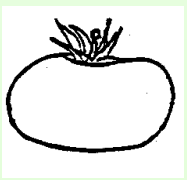
10.pyriform

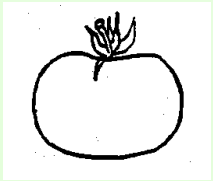
11.obcordate

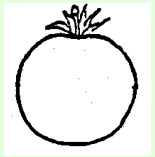
PQ: clear difference



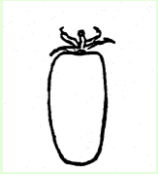



1.flattened

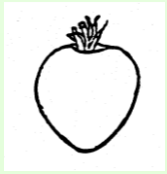

2.oblate


3.circular


4.oblong


5.cylindric

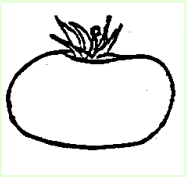

6.elliptic

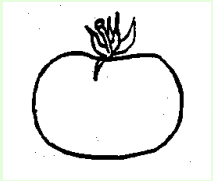

7.cordate

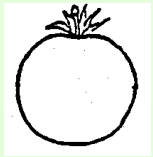

8.ovate

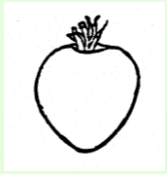

9.obovate

PQ: clear difference







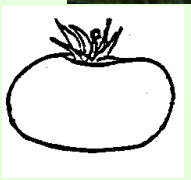


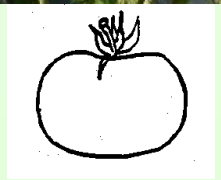
1.flattened 2.oblate 3.circular 4.oblong 5.cylindric 6.elliptic 7.cordate 8.ovate 9.obovate

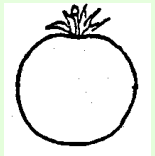
PQ: clear difference







1.flattened

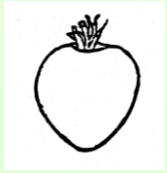

2.oblate


3.circular

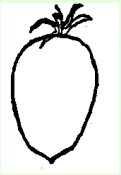

4.oblong


5.cylindric

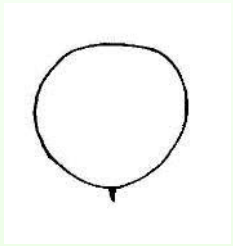

6.elliptic


7.cordate

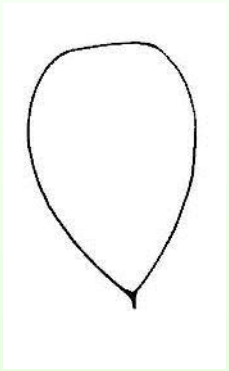

8.ovate


9.obovate

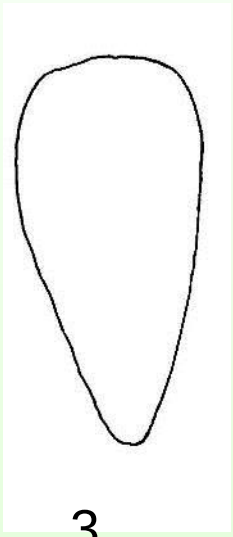
10. **(*)** **(+)**	**VG**	**Root: shape in longitudinal section**	**TG/49/7 Carrot**	
PQ	**(b)**	**circular**	**Parijse Markt 2, Parijse Markt 3**	**1**
		obovate		**2**
		medium obtriangular	**Chantenay, De Colmar à cœur rouge 2**	**3**
		narrow obtriangular	**Imperator, De Colmar à cœur rouge 3**	**4**
		narrow obtriangular to narrow oblong	**Maestro**	**5**
		narrow oblong	**Amsterdam 2, Berlikumer 2,**	**6**



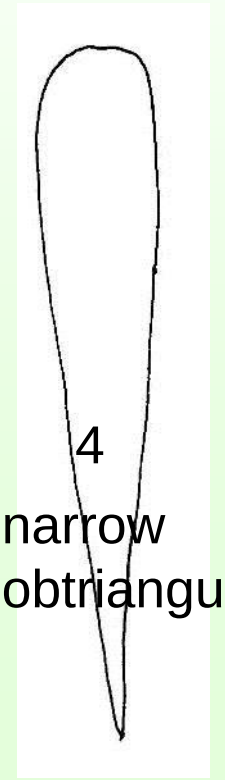
1
circular



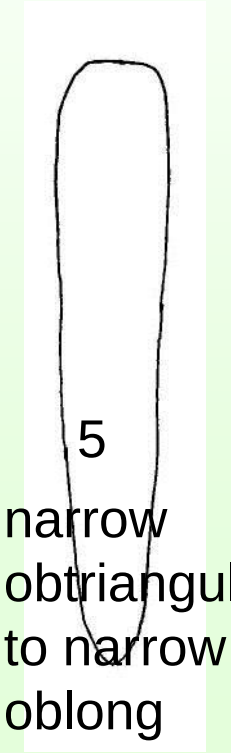
2
obovate



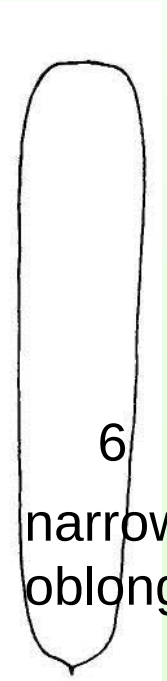
3
medium
obtriangular



4
narrow
obtriangular

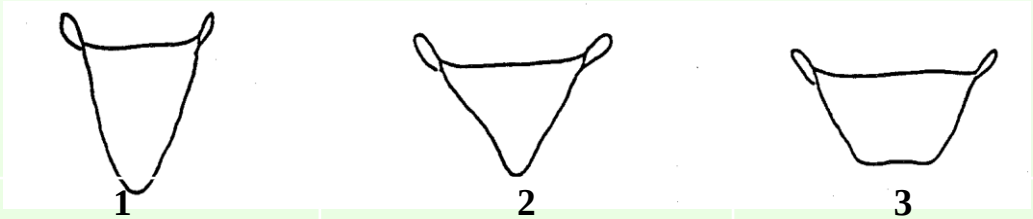


5
narrow
obtriangular
to narrow
oblong

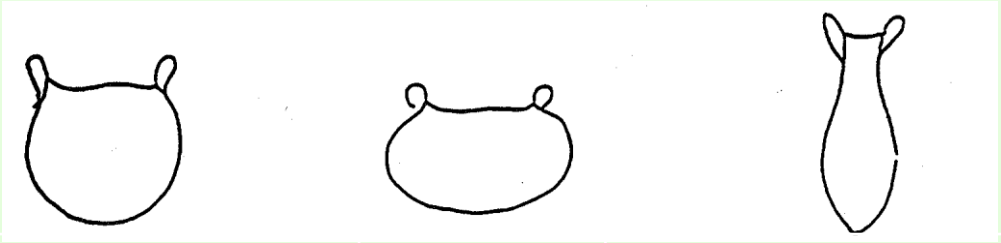


6
narrow
oblong

Cymbidium:
Lip: shape



1	2	3
narrow triangular	triangular	trapezium



4	5	6
circular	oblate	spatulate

QN

Distinctness examination

- Transfer from measured value to note
- Two note rule

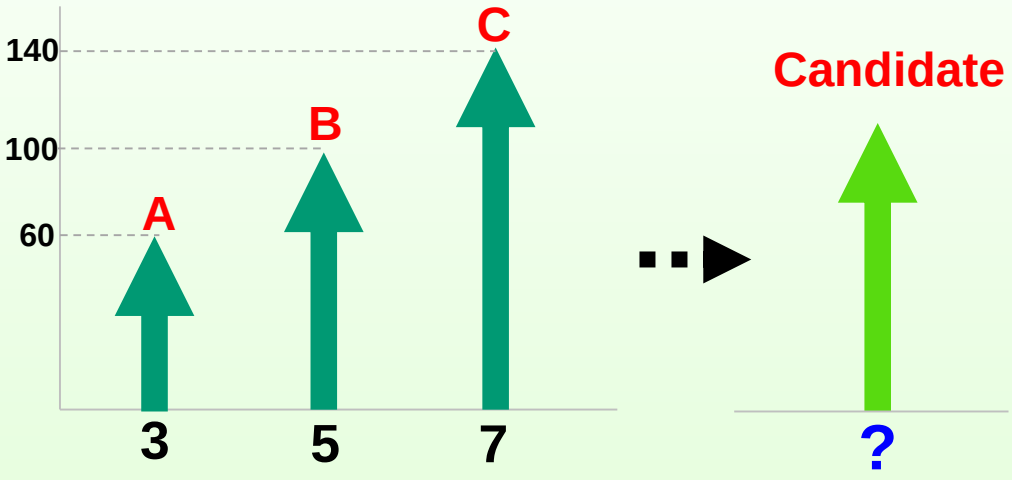
Distinctness

Clear difference

- **transfer from measured value to note**

TG/2/7 Maize

24.2 (*) (+)	MS	Plant: length	Example varieties	note	cm (example)
QN		very short		1	
	75-85	short	PR39D23 (A)	3	60
		medium	PR37Y12 (B)	5	100
		long	DKC5166 (C)	7	140
		very long		9	
			Candidate	?	115



Distinctness

Clear difference

■ Note setting table

- ✓ “60”, “140” are middle value in the range of Note3, Note7
- ✓ width of one note → (140-60) / (7-3) = 80/4 = 20cm

notes	1	2	3	4	5	6	7	8	9
			60		←20→		**140**		

A **C**

Distinctness

Clear difference

Note setting table

✓ **starting point of Note3 → 60- 20/2 = 60-10 = 50**

notes	1	2	3	4	5	6	7	8	9
			←20→						
			60				**140**		
			A				**C**		

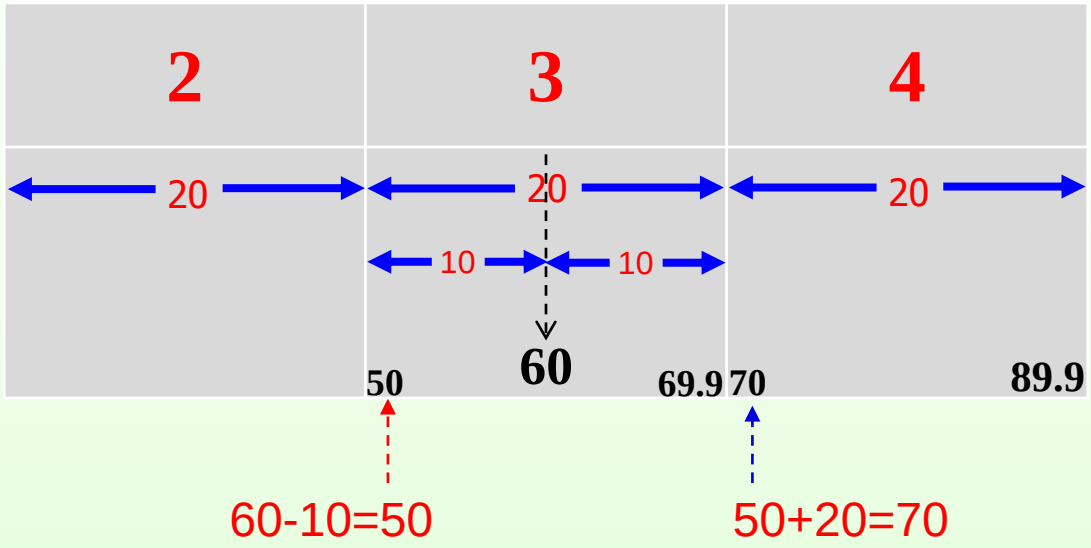
60-10=50

50+20=70

notes	1	2	3	4	5	6	7	8	9
interval	**~ 10**	**30 ~**	**50 ~**	**70 ~**	**90 ~**	**110 ~**	**130 ~**	**150 ~**	**170 ~**
			A				**C**		

Candidate **115 cm** ·····▸ **note 6**

width of one note = 20cm



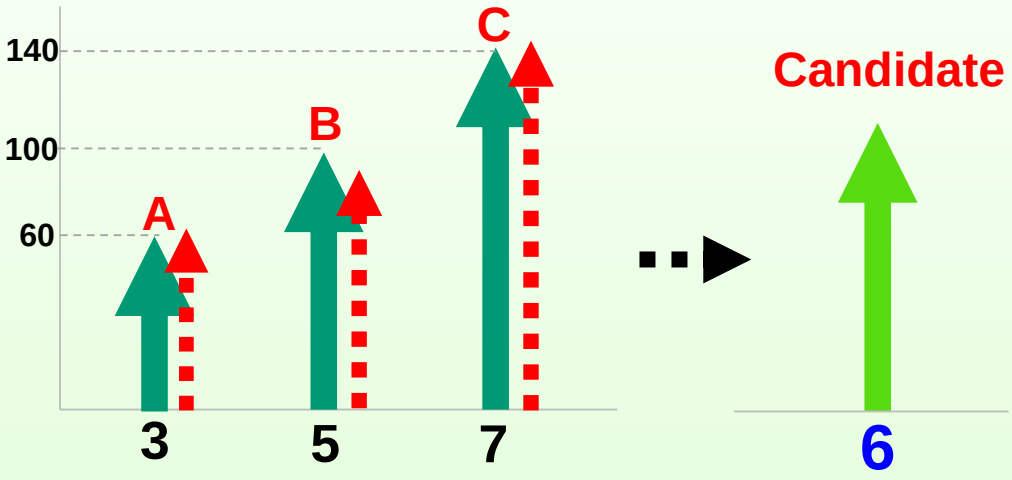
Distinctness

Clear difference

- **transfer from measured value to note**

TG/2/7 Maize

24.2 (*) (+)	MS 75-85	Plant: length	Example varieties	note	cm (example)
QN		very short		1	
		short	PR39D23 (A)	3	60
		medium	PR37Y12 (B)	5	100
		long	DKC5166 (C)	7	140
		very long		9	
			Candidate	6	115



Distinctness

Clear difference

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**

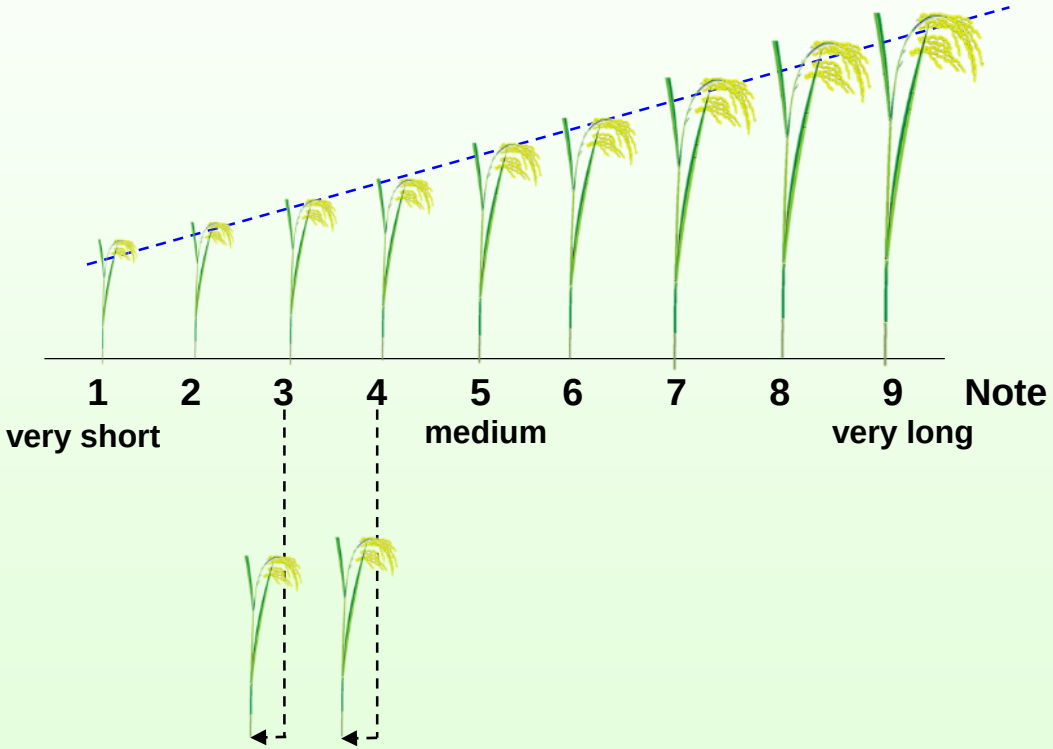
Distinctness

Clear difference

- **Two notes rule**

TG/16/8 Rice

26 (*) QN	70 VS	Stem length		
		very short	Lampo, Leda	1
		short	Loto, Thaibonnet	3
		medium	Ariete, Bahia	5
		long	Baldo	7
		very long	Carnaroli	9



Note 3: 4 → may NOT be clear difference

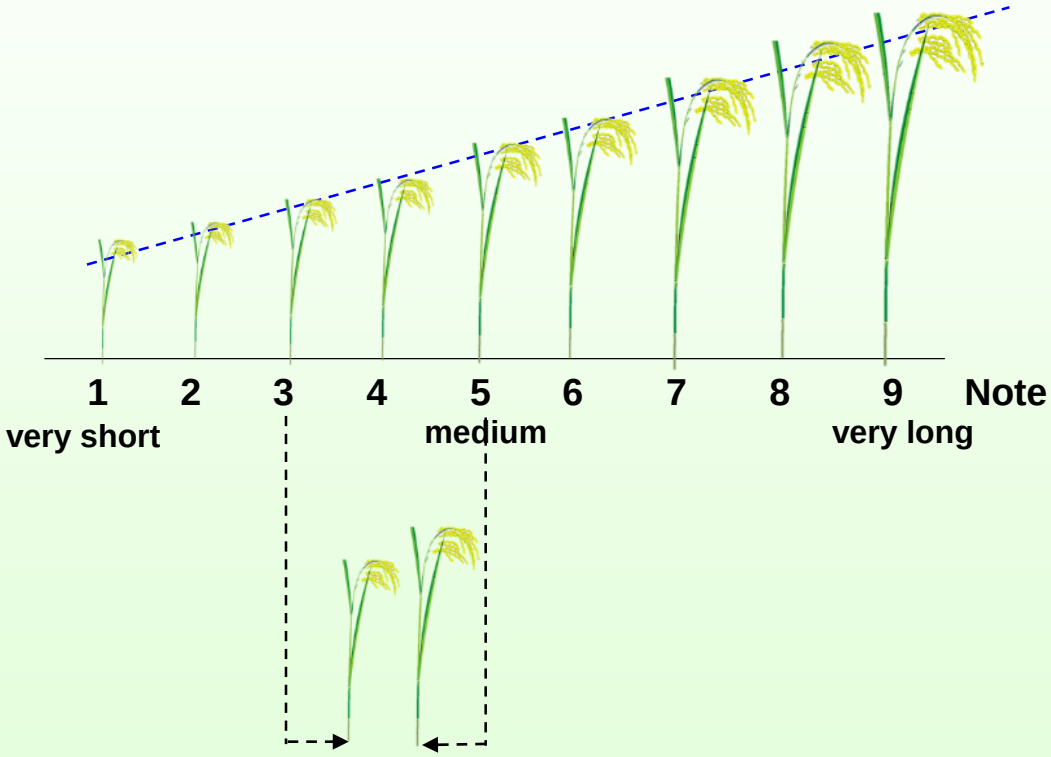
Distinctness

Clear difference

- Two notes rule

TG/16/8 Rice

26 (*) QN	70 VS	Stem length		
		very short	Lampo, Leda	1
		short	Loto, Thaibonnet	3
		medium	Ariete, Bahia	5
		long	Baldo	7
		very long	Carnaroli	9



Note 3: 5 → may be clear difference

Distinctness

Clear difference

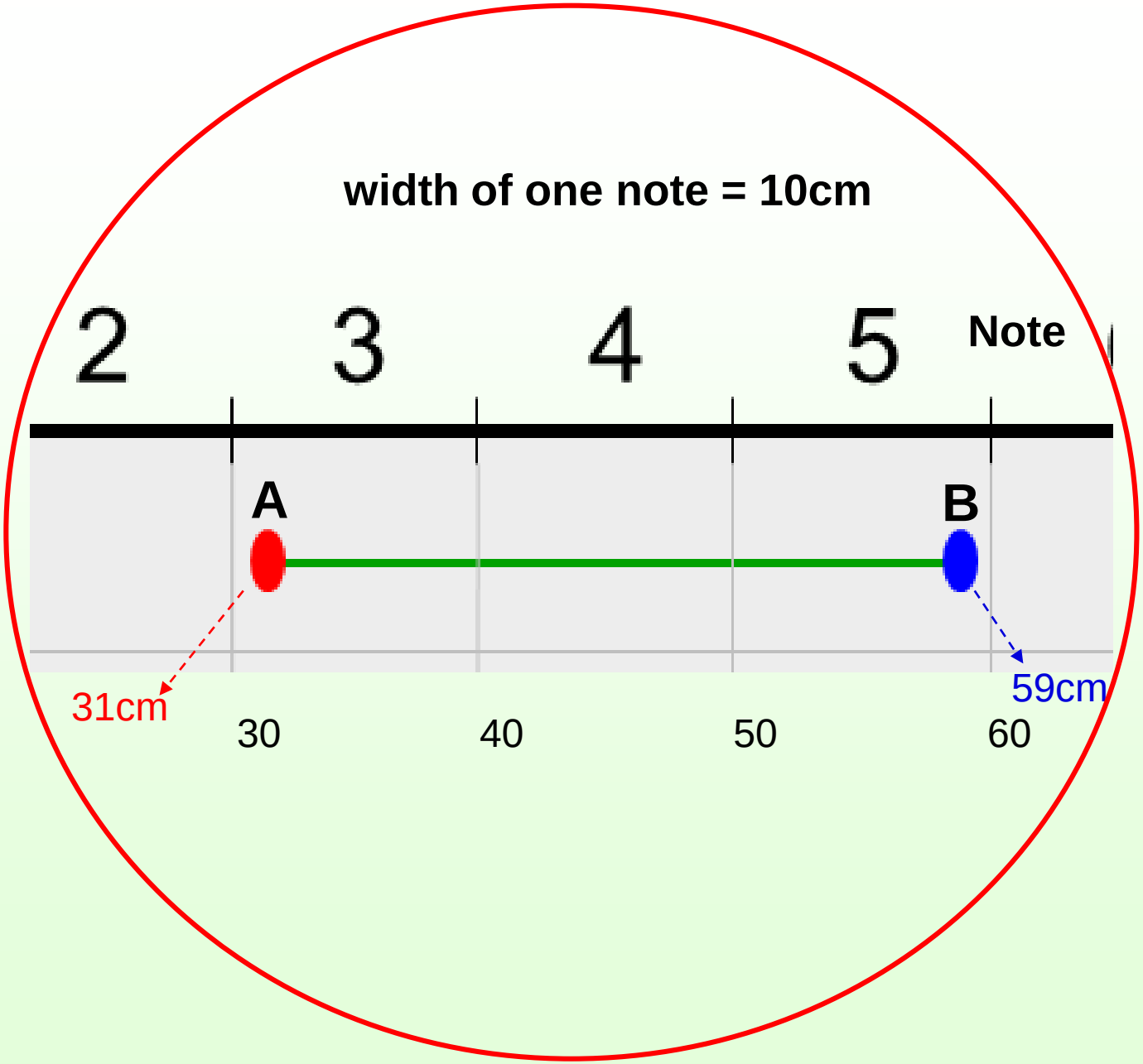
“a difference of two Notes often represents a clear difference”

“Two Note” rule

QN chars. (Length, Height)

	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	?

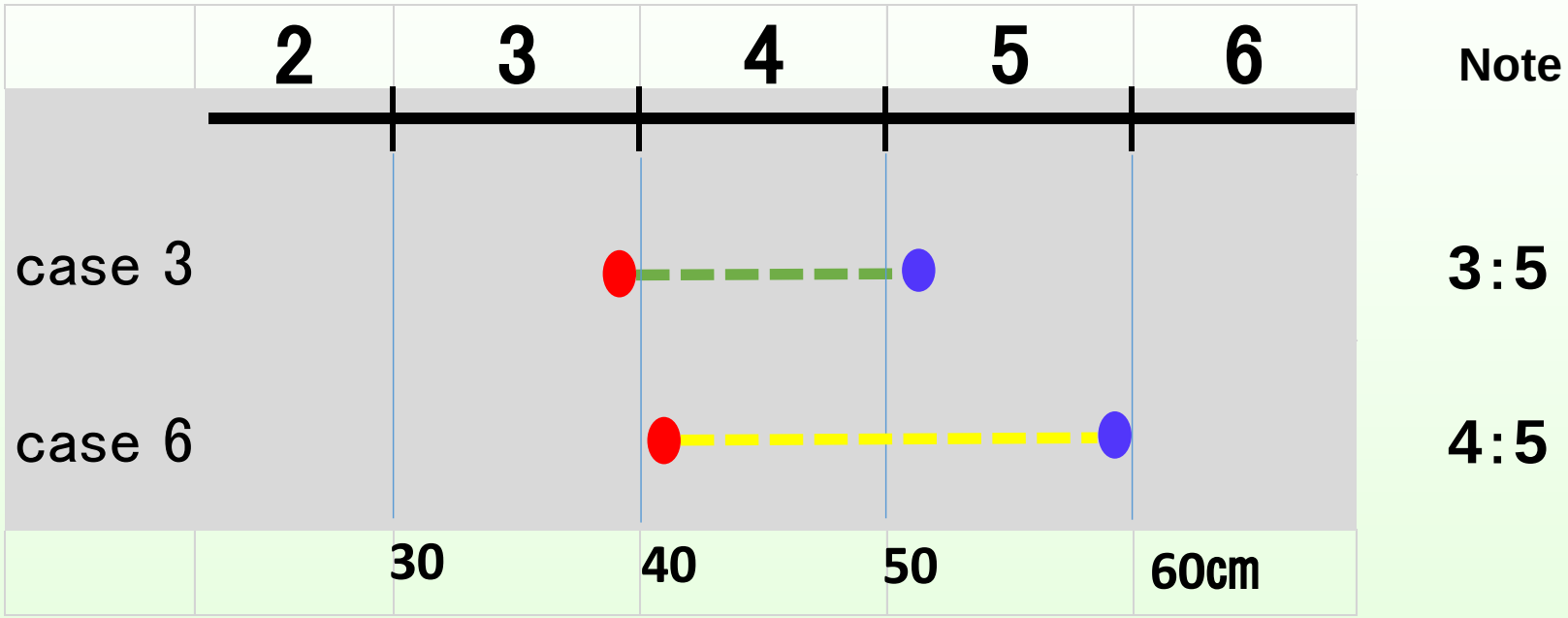
Case 1



Distinctness

Clear difference

QN: Stem: Length



distance: case 3 < case 6

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

QN distinctness = <u>" > width of one note difference"</u>

Distinctness

Clear difference

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

Distinctness

■ 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.

Uniformity examination

- **Features of propagation of the variety**
- **Off-type approach**
- **Standard Deviation approach**

Uniformity

Requirement:

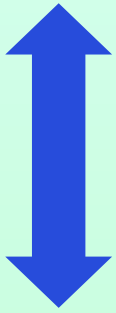
Article 8; 91 Act of the UPOV

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

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

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

TGP/10/1 2.5.2

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		

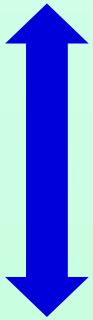
- The most common approaches are listed first.

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

Low level of genetic variation

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%

TGP/8 p.109

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**

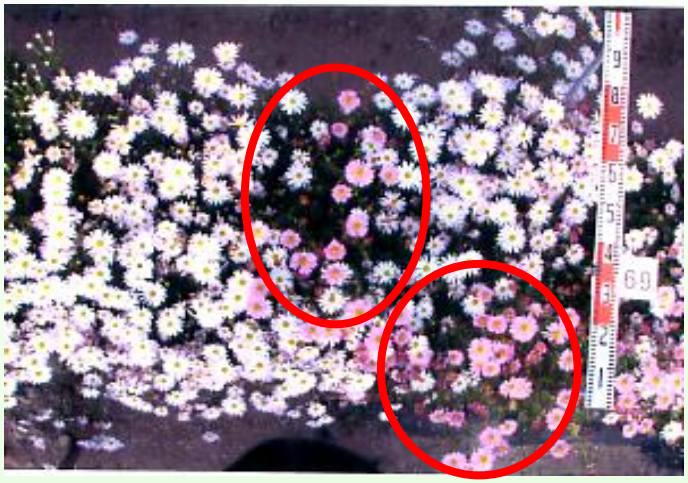
PS, AP in each UPOV TGs

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, Carnation
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

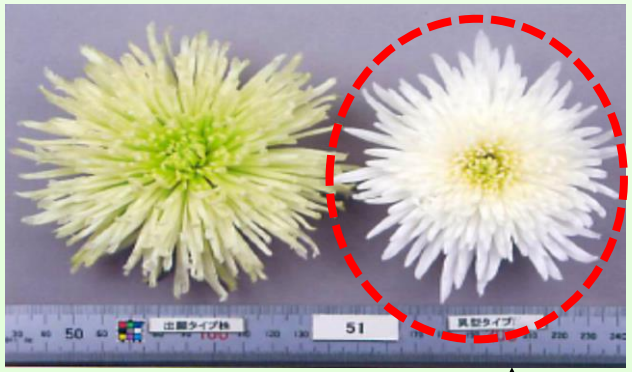
Off type

Uniformity



Off-type

Off-type



Off-type

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**

Uniformity

2. Standard deviation approach

High level of genetic variation

- ✓ used in **cross-pollinated varieties**, and for **QN : MS**

- ✓ **Cross-pollinated varieties**, generally exhibit wider variations within the variety than vegetatively propagated or self-pollinated varieties, and it is more difficult to determine off-types.

- ✓ In this case the uniformity can be assessed by considering the overall **level of variation**, observed across all the individual plants, to determine whether it is similar to comparable varieties

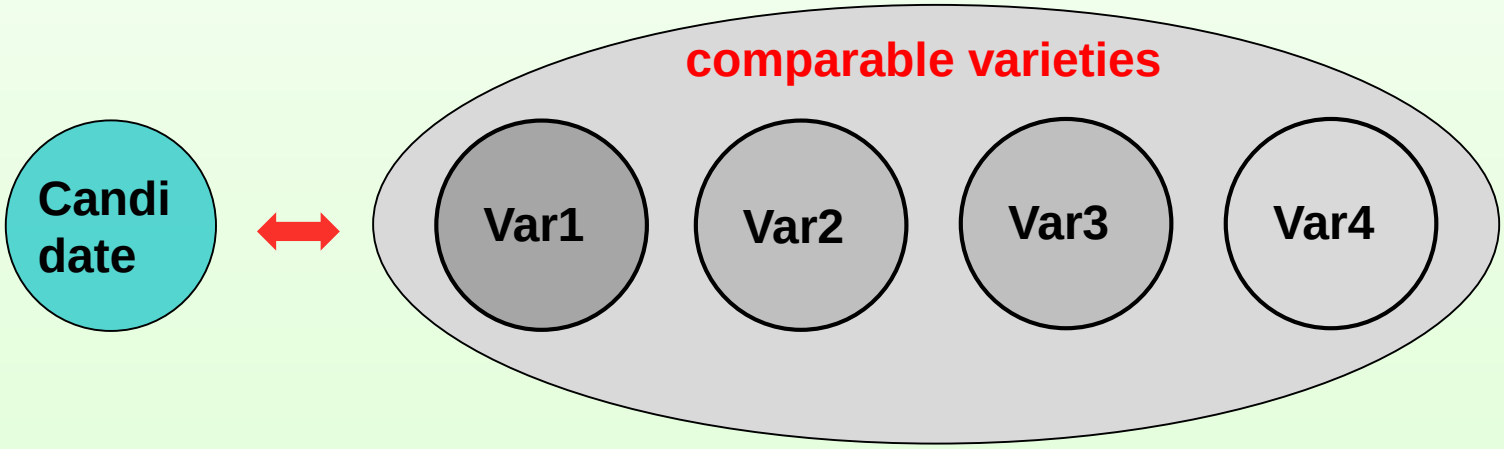
- ✓ **relative tolerance limits for the level of variation are set by comparison with comparable varieties, or types, already known ("standard deviation approach").**

Uniformity

2. Standard deviation approach (cont.)

- **Determining the acceptable level of variation**

  ✓ **The comparison between a candidate variety and comparable varieties is carried out on the basis of standard deviation (SD), calculated from individual plant observation.**



SD (A) ⟷ **Average SD (B)**

Relative SD = A / B

Uniformity

2. Standard deviation approach (cont.)

Example: TGP/8 10.1 Use of the relative variance method

variances of candidate and comparable varieties for plant height data (QN, MS)

Candidate variety	Comparable variety 1	Comparable variety 2	Comparable variety 3	Comparable variety 4
5.6	7.8	4.5	3.2	5.8

- ✓ The number of observations per variety: 60
- ✓ The average variance for comparable varieties is (7.8 + 4.5 + 3.2 + 5.8) / 4 = 5.32
- ✓ **Relative variance** = variance of the candidate / average variance of the comparable varieties = 5.6/5.32 = 1.05
- ✓ From F-table, for a sample size of 60 : ∞; the threshold = 1.47;
- ✓ Relative variance: 1.05 < 1.47
- ✓ therefore, we can conclude that **the candidate variety is sufficiently uniform for that characteristic**

if the relative variance exceeds the threshold, the candidate variety will be deemed to be non-uniform for that characteristic.

Threshold limit for relative variance and SD for some different sample sizes

Sample size of candidate	Threshold limit for relative variance (S^2)	Threshold limit for relative SD ($\sqrt{S^2}$)
30	1.70	1.30
40	1.59	1.26
50	1.53	1.24
60	1.47	1.21
80	1.41	1.19
100	1.36	1.17
150	1.29	1.14
200	1.25	1.12

TGP/8/3: PART II: 10: UNIFORMITY ASSESSMENT ON THE BASIS OF THE RELATIVE VARIANCE METHOD , P.125-126

Uniformity

Calculation of Variance

Variance formula: $s^2 = \frac{\sum_{i=1}^{n}(xi - \overline{x})^2}{n}$

Variety A
Leaf length: cm

1	2	3	4	5	6	7	8	9	10
4.2	6.7	7.3	7.5	8	8.5	8.7	8.8	9.2	9.3

n=10
Average = 7.82cm

{(4.2-7.82)^2 + (6.7-7.82)^2 + ・・・・・ + (9.2-7.82)^2 + (9.3-7.82)^2} / 10 = 2.11

Excel: =VAR.P(a:b)

SD = square root of Variance = $\sqrt{s^2}$

S

Stability examination

Stability

Requirement:

Article 9; 91 Act of the UPOV

- 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

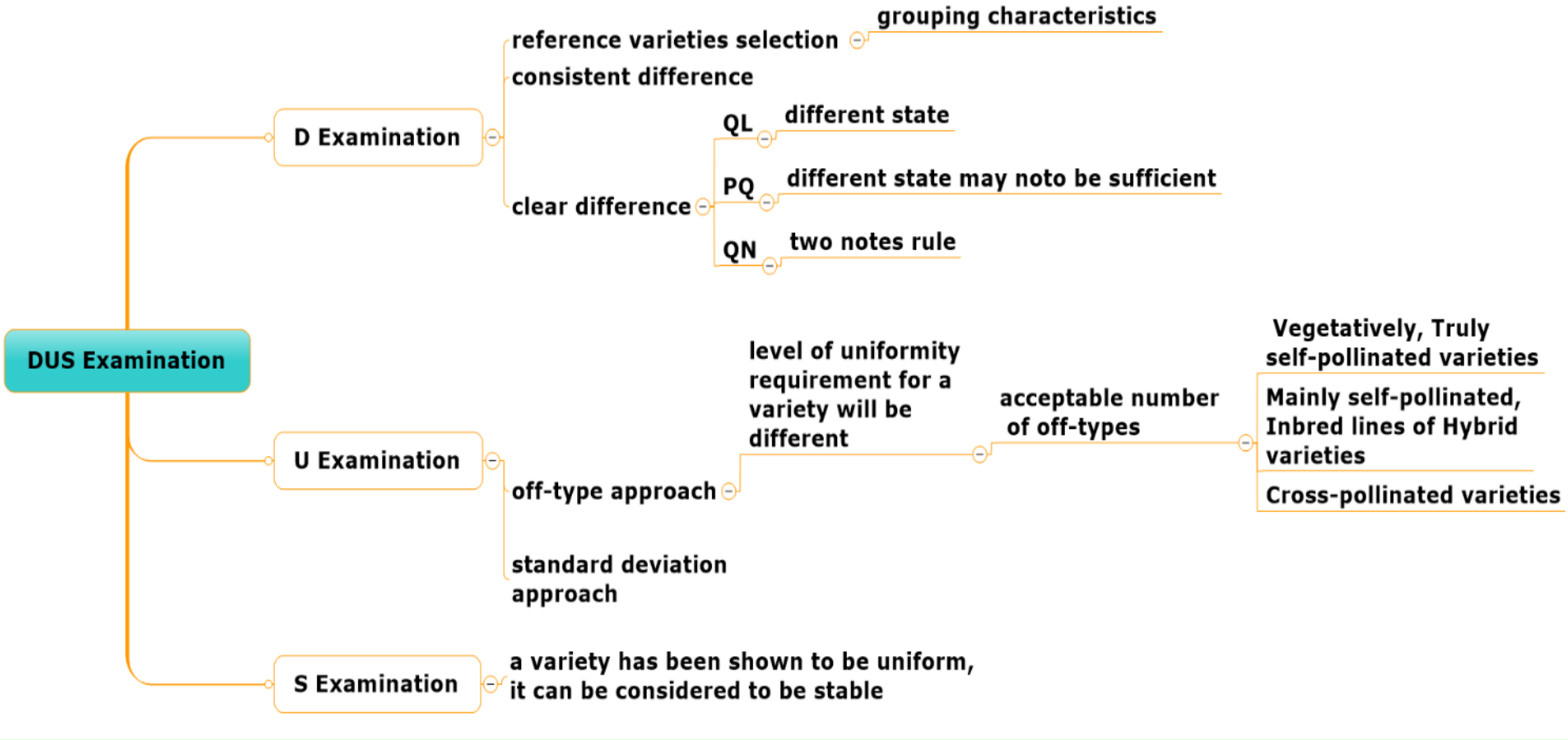
Stability

- 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.

Sammary


DUS Examination
D Examination
reference varieties selection
grouping characteristics
consistent difference
clear difference
QL
different state
PQ
different state may noto be sufficient
QN
two notes rule
U Examination
off-type approach
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
standard deviation approach
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