DUS Examination

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DUS Examination

Contents:

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

Part 1: for Vegetatively propagated, Self-pollinated varieties

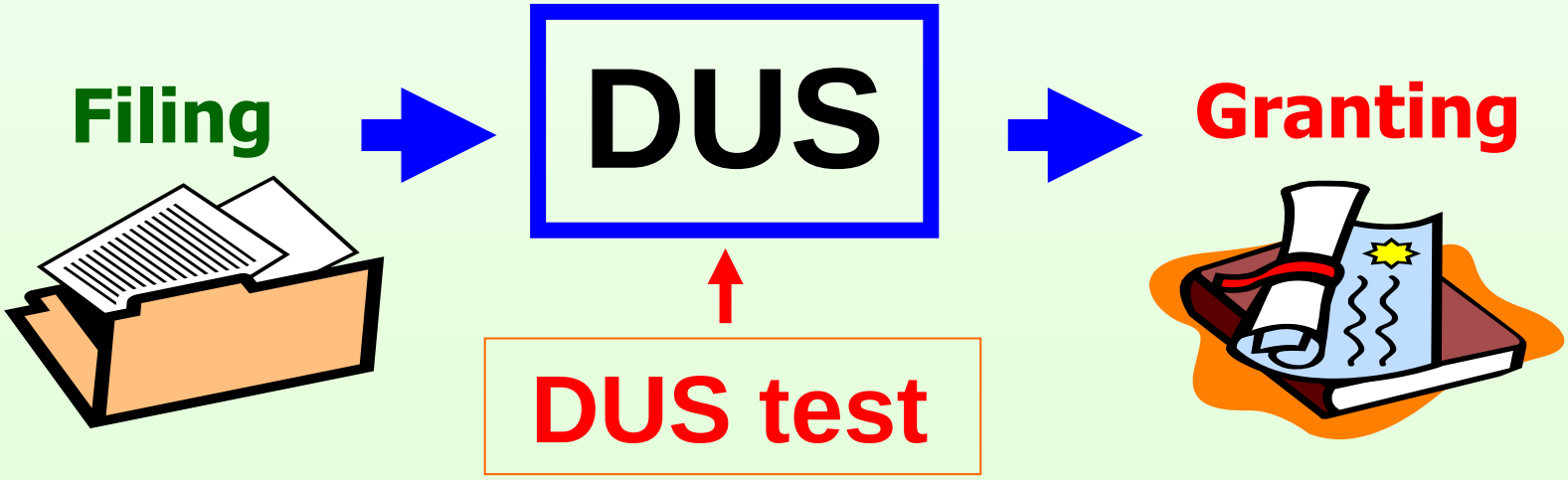
Part 2: for Cross-pollinated varieties

UPOV principles

Examination of the Application

Article 12; 91 Act of the UPOV

Any decision to grant a breeder's right shall require an examination for compliance with the conditions under article 5 to 9.



UPOV principles

D: must be distinguishable from any other varieties

U: must be uniform

S: must be unchanged after repeated propagation

Purpose of DUS test

■ **Characteristics as the Basis for Examination of DUS** TG/1/3: 2.4

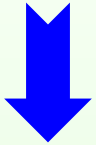
1. **For any variety to be capable of protection *it must first be clearly defined*.**
2. **Only after a variety has been defined *can it be finally examined for fulfillment of the DUS criteria* required for protection.**
3. **a *variety is defined by its characteristics* and that those characteristics are therefore the basis on which a variety can be examined for DUS.**

purpose of DUS test

1. **Definition of a variety using the characteristics**
2. **Examination of DUS**

Definition of a variety

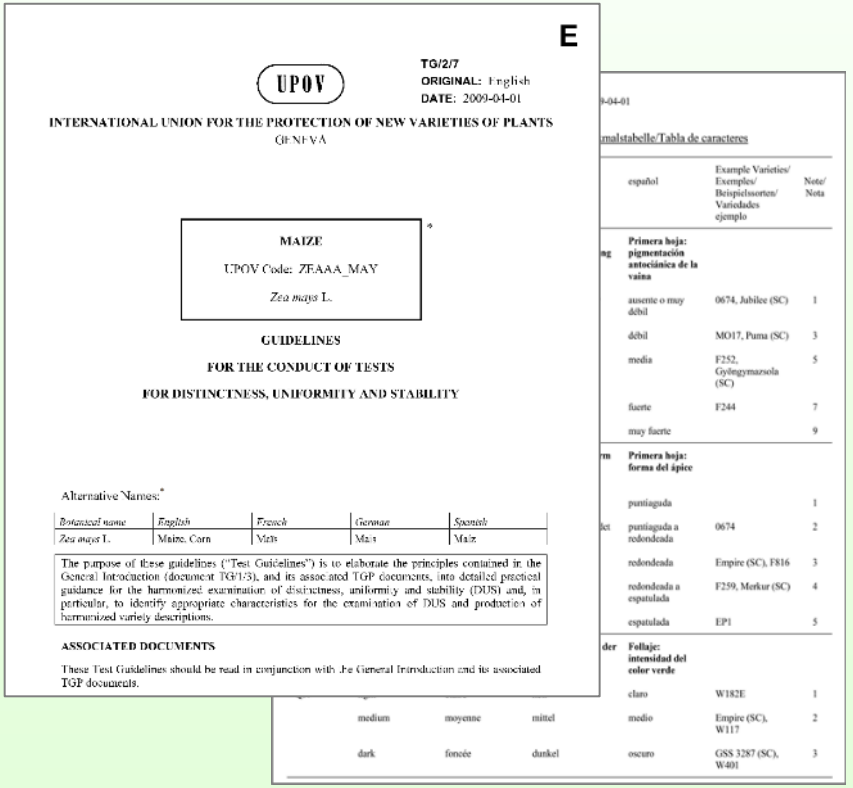
Definition of a variety



Clarify the states of expression of the characteristics of the variety and make a "variety description" of the variety using its relevant characteristics.

Definition of a variety

- How to define the variety



E

UPOV

TG/2/7
ORIGINAL: English
DATE: 2009-04-01

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

MAIZE

UPOV Code: ZEAAA_MAY

Zea mays L.

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

Alternative Names:*

Botanical name	*English*	*French*	*German*	*Spanish*
Zea mays L.	Maize, Corn	Maïs	Mais	Maíz

The purpose of these guidelines ("Test Guidelines") is to elaborate the principles contained in the General Introduction (document TG/1/3), and its associated TGP documents, into detailed practical guidance for the harmonized examination of distinctness, uniformity and stability (DUS) and, in particular, to identify appropriate characteristics for the examination of DUS and production of harmonized variety descriptions.

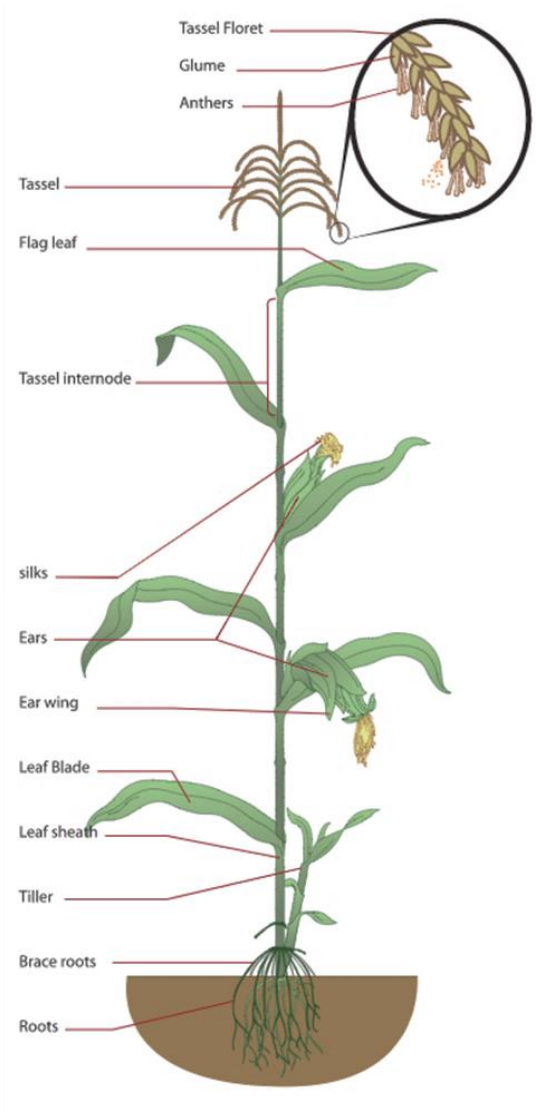
ASSOCIATED DOCUMENTS

These Test Guidelines should be read in conjunction with the General Introduction and its associated TGP documents.

malstabelle/Tabla de caracteres

	español	Example Varieties/ Exemples/ Beispielsorten/ Variedades ejemplo	Note/ Nota
	Primera hoja: pigmentación antociánica de la vaina		
	ausente o muy débil	0674, Jubilee (SC)	1
	débil	MO17, Puma (SC)	3
	media	F252, Gyöngymazsola (SC)	5
	fuerte	F244	7
	muy fuerte		9
	Primera hoja: forma del ápice		
	puntiaguda		1
	puntiaguda a redondeada	0674	2
	redondeada	Empire (SC), F816	3
	redondeada a espatulada	F259, Merkur (SC)	4
	espatulada	EP1	5
	Follaje: intensidad del color verde		
	claro	W182E	1
medium	moyenne	mittel	medio	Empire (SC), W117	2
dark	foncée	dunkel	oscuro	GSS 3287 (SC), W401	3

Maize: 41 characteristics



3

7

10. (+)	Tassel: anthocyanin coloration of glumes excluding base	Note
QN	absent or very weak	1
	weak	3
	medium	5
	strong	7
	very strong	9

14. (*)	Tassel: number of primary lateral branches	Note
QN	absent or very few	1
	few	3
	medium	5
	many	7
	very many	9

15. (+)	Ear: time of silk emergence	Note
QN	early	3
	medium	5
	late	7

Definition of a variety

■ Variety description

total 41 chars.

Char No.	Characteristics	Notes
1	First leaf: anthocyanin coloration of sheath	5
2	First leaf: shape of apex	3
3	Foliage: intensity of green color	2
4	Leaf: undulation of margin of blade	2
5	Leaf: angle between blade and stem	3
6	Leaf: curvature of blade	3
7	Stem: degree of zig-zag	2
8	Tassel: time of anthesis	4
..	.....	..

The variety description → defined by the states of expression of the characteristics

PART 1:

DUS Examination for Vegetatively propagated, Self-pollinated and Single-Cross Hybrid varieties

D

Distinctness examination

- **consistent difference**
- **Clear difference**

Distinctness Examination

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

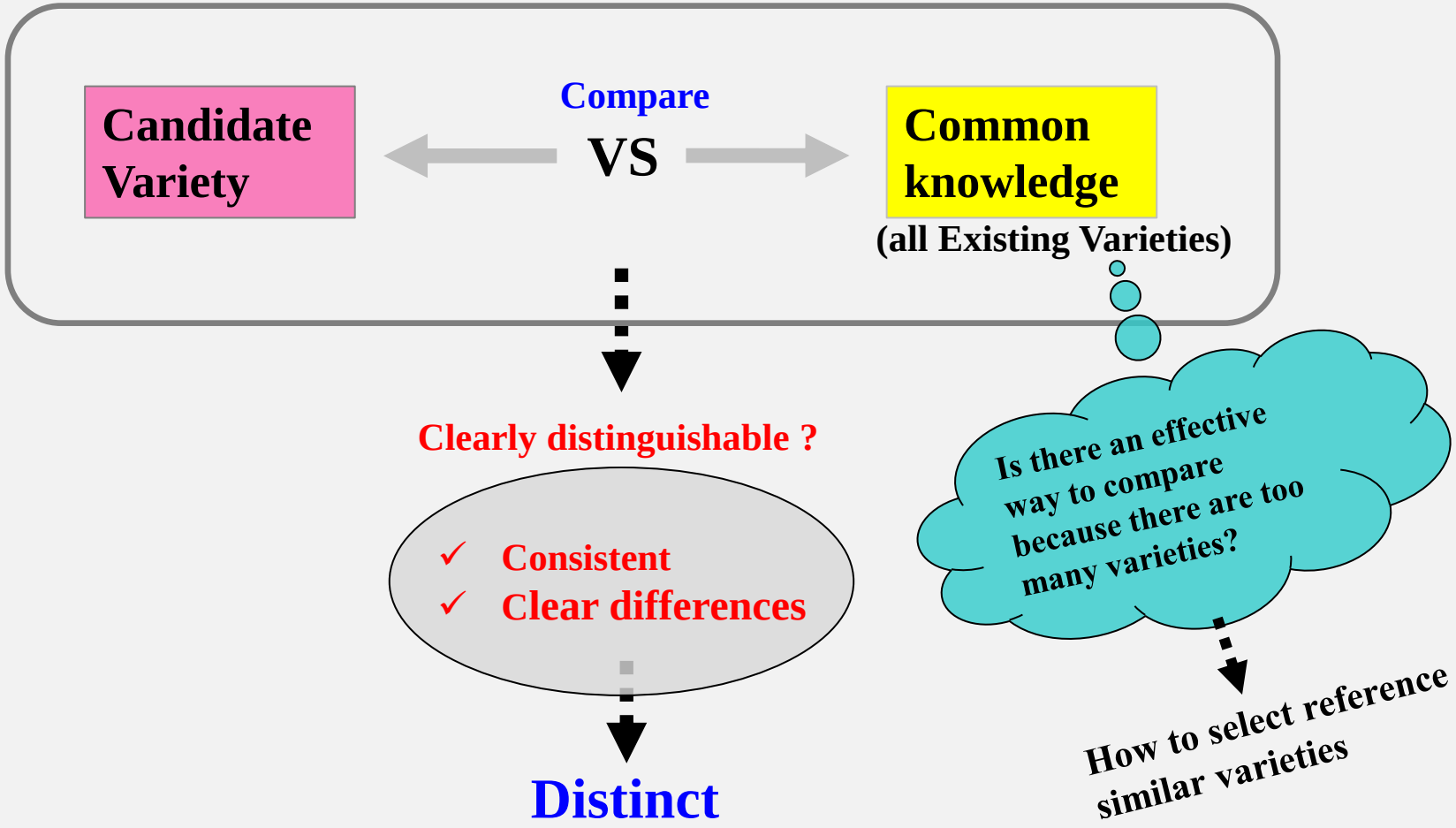
- **A variety may be considered to be clearly distinguishable if the difference in characteristics is:**

  (a) consistent, and

  (b) clear.

Distinctness Examination

D: Clearly distinguishable



Selection of reference similar variety

Selection of Similar Varieties

TG/1/3: 5.3.1.1

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

VS

We need to compare to all 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

Selection of Similar Varieties

VS

No need to compare candidate variety with different group of varieties

Selection of Similar Varieties

Selecting the similar varieties

Candidate varieties

Grouping characteristics
(Flower: predominant color)

Similar varieties

Selection of Similar Varieties

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

VS

Selection of Similar Varieties

Candidate varieties

Distinct

Distinct

Distinct

Similar varieties

the candidate variety is considered to be distinguished from all existing 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)

Grouping characteristics

Grouping Characteristics

TGP/7 5.2

Function:

A) to select varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness; and

B) to organize the growing trial so that similar varieties are grouped together.

Distinctness Examination

Consistent difference:

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

stem: length

growing season 1

Similar **Candi.**

growing season 2

Similar **Candi.**

Distinctness Examination

Consistent difference

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

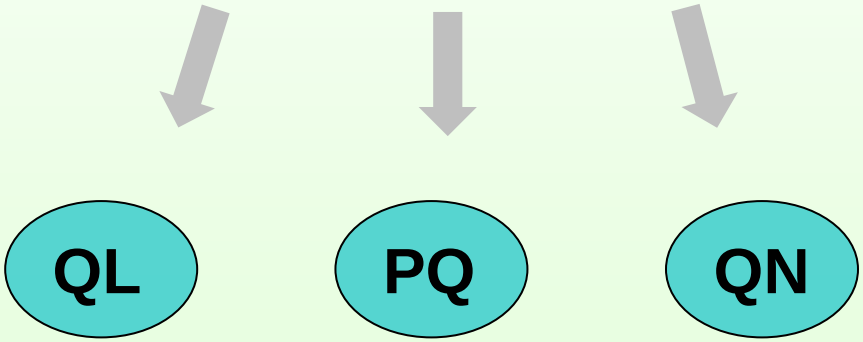
Distinctness Examination

Consistent difference

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

Distinctness examination

Distinctness: QL

Clear difference

TG/1/3： 5.3.3.2.1

QL

- Visual observation
- 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: QL

Clear difference

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

Fruit : green shoulder (before maturity)

Absent 1 Present 9

Peduncle: abscission layer

http://www.johnnyseeds.com https://www.deruiterseeds.com

Absent 1 Present 9

Distinctness: QL

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

zygomorphy 1

https://www.anniesannuals.com

actinomorphy 2

PQ

Distinctness examination

Distinctness: PQ

Clear difference

TG/1/3： 5.3.3.2.3

PQ

- **Visual observation**
- **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.

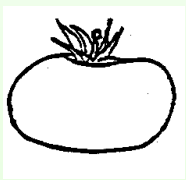
✓ difficult to define a general rule on the difference in Notes to establish Distinctness

✓ need to compare the state of expression directly side by side with observing many samples

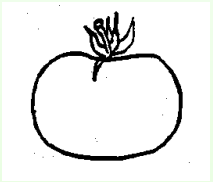
Distinctness: PQ

Clear difference

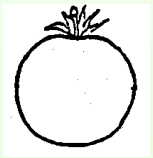
PQ: clear difference



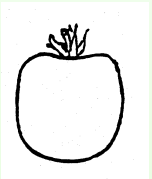
1.flattened



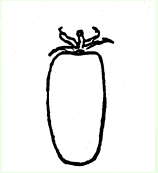
2.oblate



3.circular

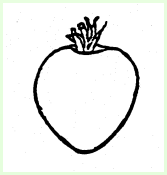


4.oblong



5.cylindric

6.elliptic



7.cordate

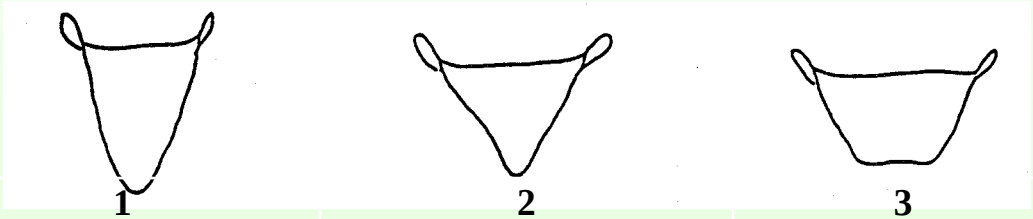
8.ovate

9.obovate

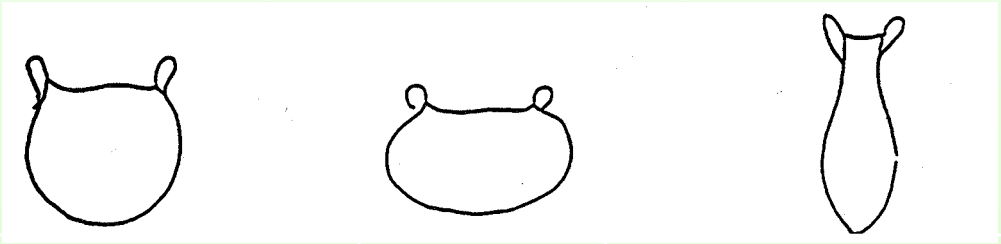
10.pyriform

11.obcordate

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: QN

Clear difference

QN

TG/1/3： 5.3.3.2.2

- **Visual observation / measurement**
- **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.

✓ **“Two Notes” rule**

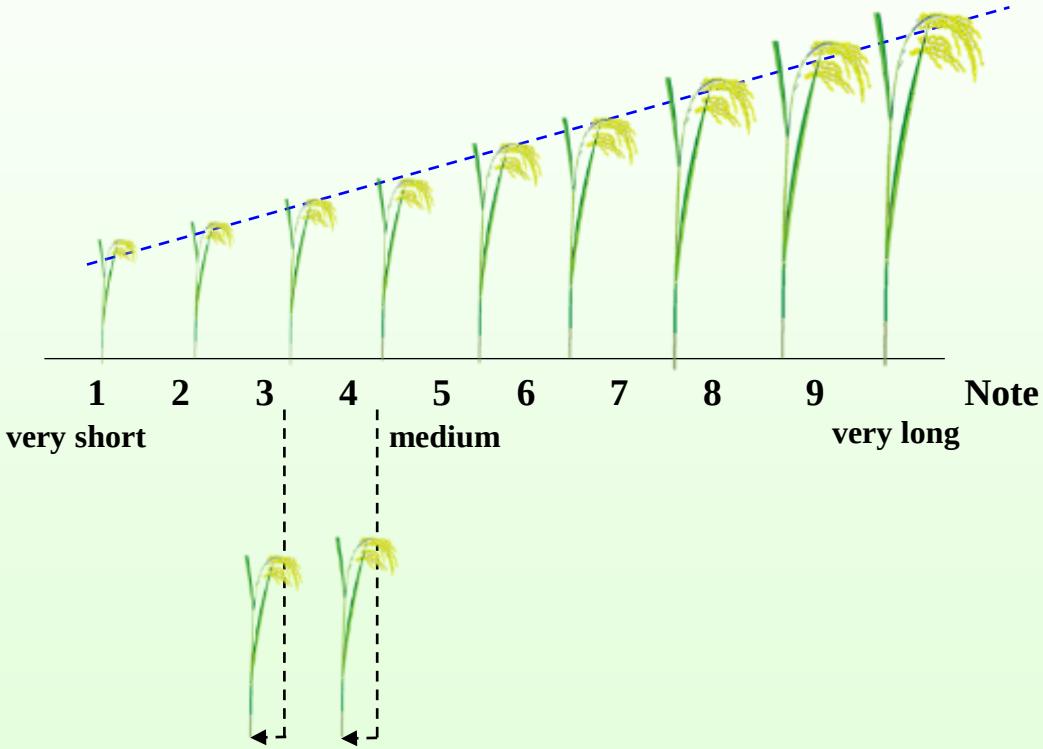
Distinctness: QN

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

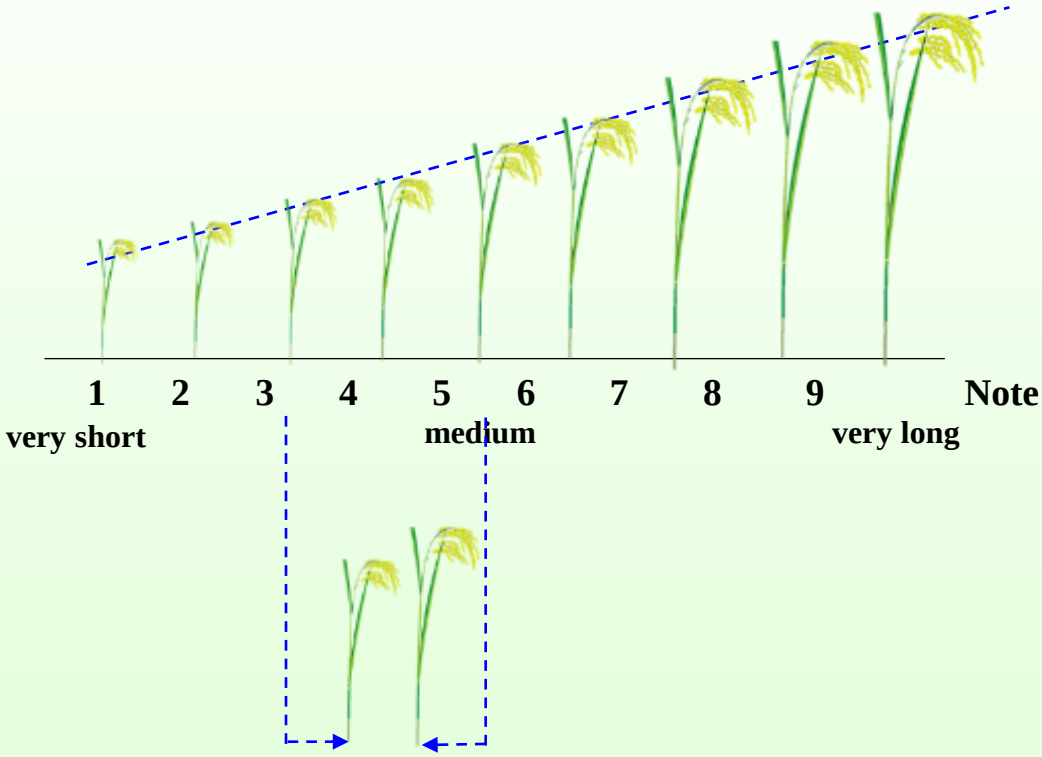
Distinctness: QN

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: QN

Clear difference

- **How to examine Distinctness in QN**
  - ✓ **How do we get a "Note" from measured value ?**
  - ✓ **How do we evaluate the difference between candidate variety and similar varieties?**

(example) " Plant: length" **(MS)**

Varieties	length cm	Notes	Distinctness
Candidate	115	?	
Sim. 1	91	?	D or not D?
Sim. 2	98	?	D or not D?
Exa. 1	60	3	
Exa. 2	140	7	

Distinctness: QN

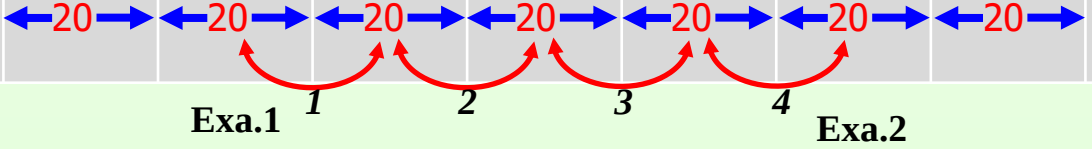
Clear difference

- **How do we get a "Note" from measured value ?**

Step1: Making a Note Setting Table

- ✓ **put “60”, “140” of Example varieties values into middle 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				140		



Exa.1 **Exa.2**

Distinctness: QN

Clear difference

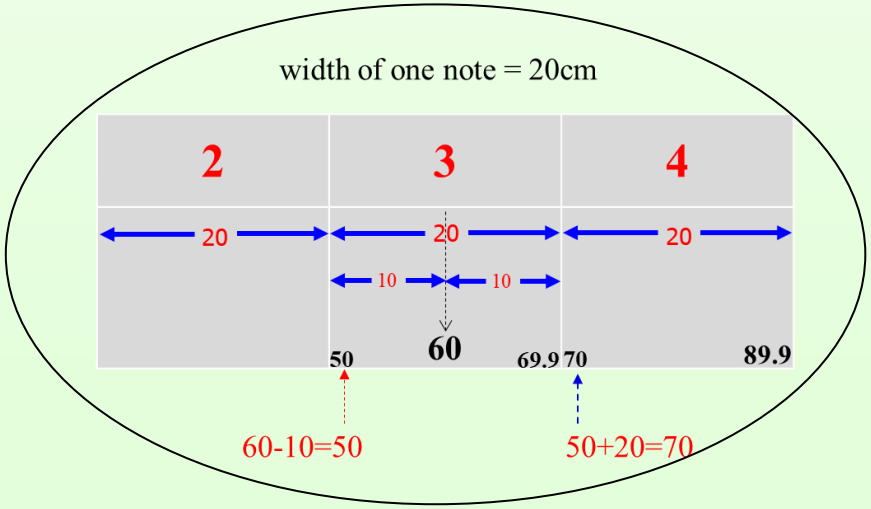
Step1: Making a Note Setting Table

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

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

60-10=50

50+20=70



Distinctness: QN

Clear difference

Step1: Making a 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**		
			Exa.1				**Exa.2**		

60-10=50

50+20=70

[Note setting table]

notes	1	2	3	4	5	6	7	8	9
interval	~ **29.9**	**30 ~ 49.9**	**50 ~ 69.9**	**70 ~ 89.9**	**90 ~ 109.9**	**110 ~ 129.9**	**130 ~ 149.9**	**150 ~ 169.9**	**170 ~**
States of Expressions			**short**		**med.**		**long**		

Distinctness: QN

Clear difference

Step2: Convert measured value to Note using the NST

[Note setting table]

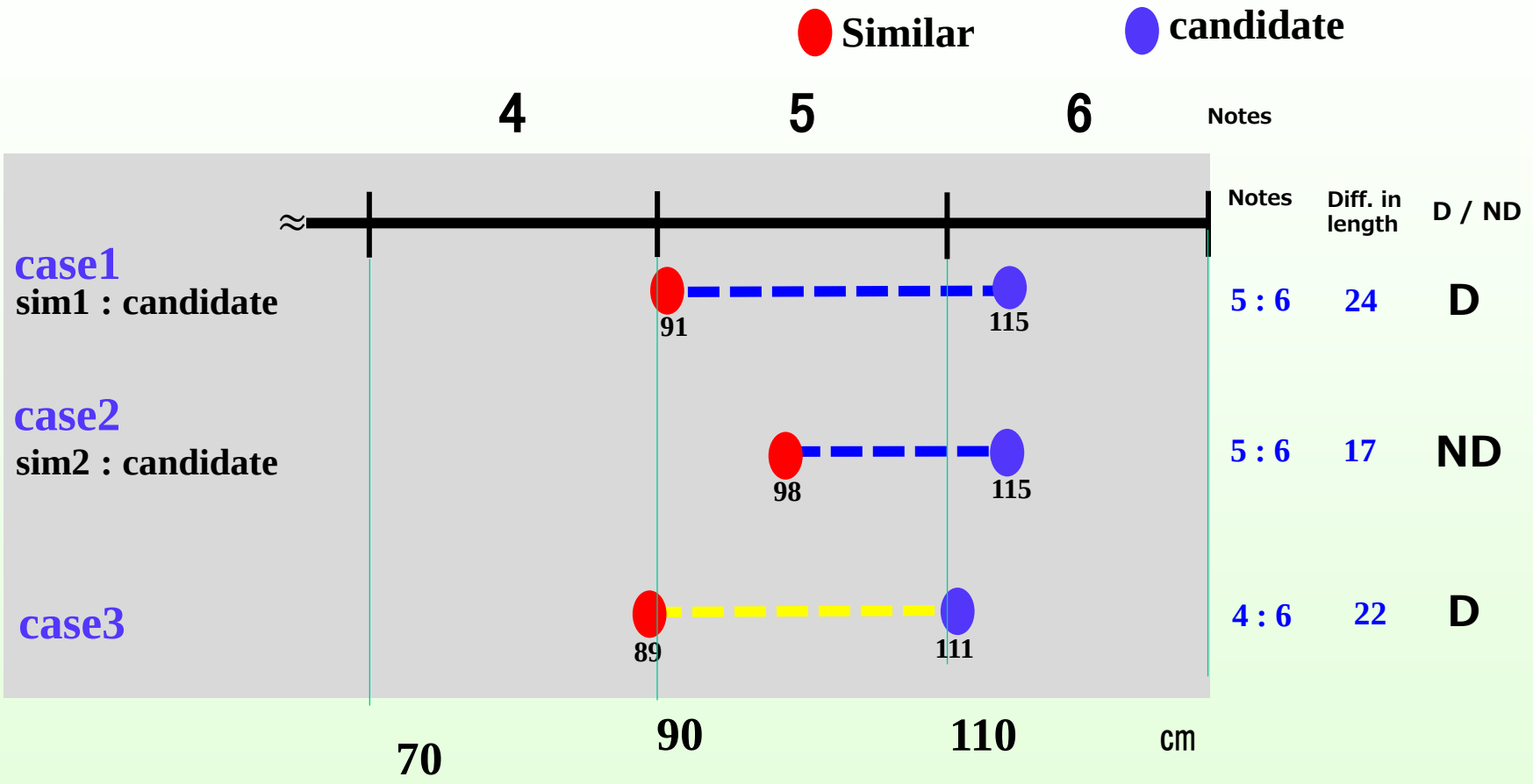
notes	1	2	3	4	5	6	7	8	9
interval	~ 29.9	30 ~ 49.9	50 ~ 69.9	70 ~ 89.9	90 ~ 109.9	110 ~ 129.9	130 ~ 149.9	150 ~ 169.9	170 ~
States of Expressions			short		med.		long		

(example) “ Plant: length” (MS)

Varieties	length cm	Notes	Distinctness		Diff. in length
Candidate	115	6			
Sim. 1	91	5	D or not D?	1 Note difference	24
Sim. 2	98	5	D or not D?	1 Note difference	17
Exa. 1	60	3			
Exa. 2	140	7			

Distinctness: QN

Clear difference



A "Two note rule" means that there is a difference of more than width of one note.

QN(MS) distinctness = " > width of one note

Distinctness: QN

Clear difference

- **Transformation of measurements into Notes**

UPOV

International Union for the Protection of New Varieties of Plants

TWC/35/12 E

Technical Working Party on Automation and Computer Programs TWC/35/12

Thirty-Fifth Session
Buenos Aires, Argentina, November 14 to 17, 2017

Original: English
Date: October 23, 2017

SHORT EXPLANATION ON THE JAPANESE METHODS FOR ASSESSMENT TABLE FOR PRODUCING VARIETY DESCRIPTIONS

Document prepared by an expert from Japan

Disclaimer: this document does not represent UPOV policies or guidance

1. The measured data for QN characteristics in DUS growing trial are transformed to numerical notes based on the assessment table. The assessment table are developed by the measurement data of respective example variety which are allocated in the specific notes, are precisely defined each range of notes. In case

Distinctness Examination

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 Examination

■ Assessment of Distinctness

Method of propagation of the variety	QL	PQ	QN
	V=Visual observation		V=Visual observation or M=Measurement
Vegetatively propagated, self-pollinated	**Notes(VG)**	**Notes(VG)** Side-by-side(VG)	**Notes (VG/MG/MS)** Side-by-side (VG) Statistics (MG/MS)
Cross-pollinated	Notes(VG) Statistics(VS*)	Notes(VG) Side-by-side(VG) Statistics(VS*)	Statistics ([MG]/MSVS) Side-by-side (VG) Notes (VG/MG/MS)
Hybrid **- Single cross**	**Notes(VG)**	**Notes(VG)** Side-by-side(VG)	**Notes (VG/MG/MS)** Side-by-side (VG) Statistics (MG/MS)

- The most common approach are listed first.

U

Uniformity examination

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

Uniformity Examination

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.**

✓ The level of uniformity required for the variety depends on the propagation method of the varieties.

Uniformity Examination

■ Assessment of 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 Examination

- **Methods for Uniformity assessment**
  1. **Off-types approach**
  2. **Standard deviation approach**

Features of propagation	Genetic variation	Uniformity assessment	Acceptable Off-types
•Vegetatively propagated	Lower	Off-types	Lower
•Self-pollinated		Off-types	
•Single Hybrid(inbred line)		Off-types	Higher
•Cross-pollinated			
•Multiple cross Hybrid	Higher	standard deviation	
		standard deviation	

Uniformity Examination

- **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 can we accept?

Uniformity Examination

How many off-types can we accept?

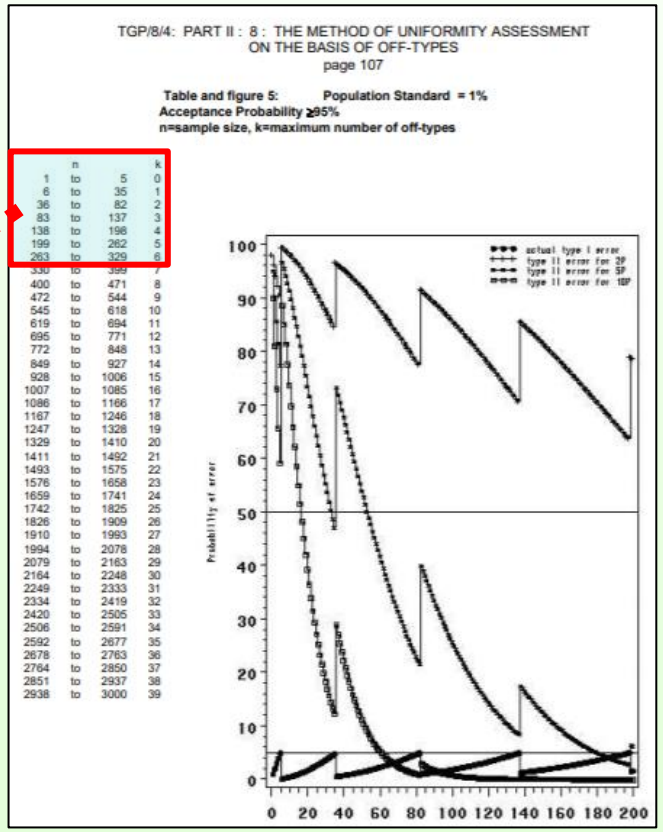
Number of off-types allowed depending on sample size

population standard = 1% and

acceptance probability = 95%

TGP/8 p.107

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



TGP/8/4: PART II : 8 : THE METHOD OF UNIFORMITY ASSESSMENT ON THE BASIS OF OFF-TYPES
page 107

Table and figure 5: Population Standard = 1%
Acceptance Probability ≥95%
n=sample size, k=maximum number of off-types

n			k
1	to	5	0
6	to	35	1
36	to	82	2
83	to	137	3
138	to	198	4
199	to	262	5
263	to	329	6
330	to	399	7
400	to	471	8
472	to	544	9
545	to	618	10
619	to	694	11
695	to	771	12
772	to	848	13
849	to	927	14
928	to	1006	15
1007	to	1085	16
1086	to	1166	17
1167	to	1246	18
1247	to	1328	19
1329	to	1410	20
1411	to	1492	21
1493	to	1575	22
1576	to	1658	23
1659	to	1741	24
1742	to	1825	25
1826	to	1909	26
1910	to	1993	27
1994	to	2078	28
2079	to	2163	29
2164	to	2248	30
2249	to	2333	31
2334	to	2419	32
2420	to	2505	33
2506	to	2591	34
2592	to	2677	35
2678	to	2763	36
2764	to	2850	37
2851	to	2937	38
2938	to	3000	39

Uniformity Examination

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 Examination

- **Population standard**

  (Acceptable Number of off-types)

  - ✓ **maximum percentage of off-types that would be permitted if all individuals of the variety could be examined. (1, 2, 3%...)**

- **Acceptance probability**

  - ✓ **Minimum probability of accepting as uniform a variety with the population standard of off-types. (95%)**

Uniformity Examination

- **Maximum number of off-types**
  (acceptance probability; 95%)

Sample size	Population Standard						
	10%	5%	3%	2%	1%	0.50%	0.10%
10	3	2	1	1	1	0	0
20	4	3	2	2	1	1	0
60	10	6	4	3	2	1	1
100	15	9	6	5	3	2	1

TGP/8/4: PART II : 8 : THE METHOD OF UNIFORMITY ASSESSMENT ON THE BASIS OF OFF-TYPES
8.1.10 Tables and figures

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 Examination

TG/1/3 6.4.1.1

Off-type

- 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**

Distinctness Requirement

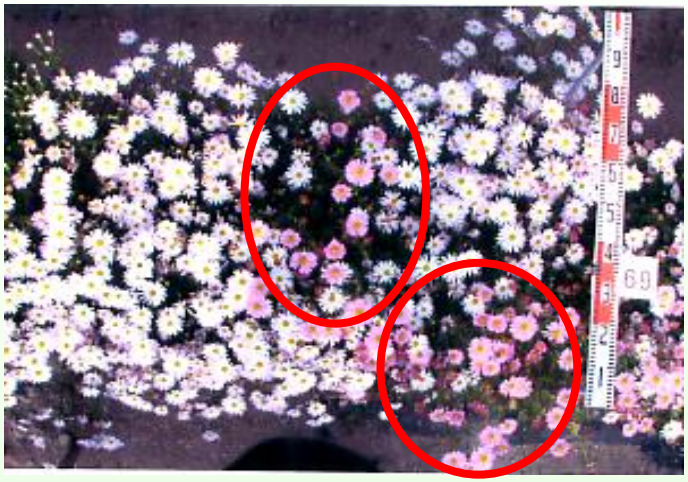
Article 7; 91 Act of the UPOV

- **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.**

Uniformity Examination

Off type

Uniformity Examination



Off-type

Off-type

Stability examination

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.
- ✓ However, when a variety has been shown to be uniform, it can be considered to be stable
- ✓ 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.

PART 2:

DUS Examination for Cross-pollinated varieties

D

Cross-pollinated varieties

Distinctness examination

- **QL, PQ => Notes approach, same as vegetative propagated, self-pollinated varieties**
- **QN => Statistical analysis / Notes approach**

↓

COYD method

Distinctness Examination

Cross-pollinated varieties

V=Visual observation

V=Visual observation or M=Measurement

Method of propagation of the variety	QL	PQ	QN
Vegetatively propagated, self-pollinated	Notes(VG)	Notes(VG) Side-by-side(VG)	Notes (VG/MG/MS) Side-by-side (VG) Statistics (MG/MS)
Cross-pollinated	**Notes(VG)** Statistics(VS*)	**Notes(VG)** Side-by-side(VG) Statistics(VS*)	**Statistics ([MG]/MSVS)** Side-by-side (VG) Notes (VG/MG/MS)
Hybrid - **Three way cross** - **Double cross**	**Notes(VG)**	**Notes(VG)** Side-by-side(VG)	**Statistics ([MG]/MSVS)** Side-by-side (VG) Notes (VG/MG/MS)

- The most common approach are listed first.

COYD method

■ **UNITED KINGDOM NATIONAL LIST/ PLANT BREEDERS RIGHTS TECHNICAL PROTOCOL FOR THE OFFICIAL EXAMINATION OF DISTINCTNESS, UNIFORMITY AND STABILITY (DUS)**

CARROT

Character Number									
CPVO TP/ CARROT/1 2004	UPOV TG/206/1 2003	UK	Character	Material examined	Number of plants or sample size for assessment	Method of assessment and recording	States of expression	D Method and Minimum distance required	U Method and Standard applied
1D QN VG	1 QN VG	60D QN VG	**Foliage: width of crown**	DUS plot	At least 400 plants in total from 4 replicates	Visual observation or visual score	3 = narrow 5 = medium 7 = broad	Clear visual difference or 2 states	Off-type standard and Uniformity score >5
2D QN VG	2 QN VG	70D QN VG	**Leaf: attitude**	DUS plot	At least 400 plants in total from 4 replicates	Visual observation or visual score	1 = erect 3 = semi-erect 5 = prostrate	Clear visual difference or 2 states	Off-type standard and Uniformity score >5
3DG QN VG/MS	*3G QN VG/MS	20D QN VG/MS	**Leaf: length (including petiole)**	DUS plot or single plants	At least 400 plants in total from 4 replicates or at least 40 plants in total from 4 replicates	Visual observation or visual score or measurements on single plants	1 = very short 3 = short 5 = medium 7 =long 9 = very long	Clear visual difference or 2 states or COYD @ 5% for both 2 and 3 year tests	Off-type standard and Uniformity score >5 or COYU at 0.1% for both 2 and 3 year tests
4D QN VG	*4 QN VG	45D QN VG	**Leaf: division**	DUS plot	At least 400 plants in total from 4 replicates	Visual observation or visual score	3 = fine 5 = medium 7 = coarse	Clear visual difference or 2 states	Off-type standard and Uniformity score >5
5D QN VG	*5 QN VG	71D QN VG	**Leaf: intensity of green colour**	DUS plot	At least 400 plants in total from 4 replicates	Visual observation or visual score	3 = light 5 = medium 7 = dark	Clear visual difference or 2 states	Off-type standard and Uniformity score >5
6D QL VG	*6 QL VG	24D QL VG	**Leaf: anthocyanin coloration of petiole**	DUS plot	At least 400 plants in total from 4 replicates	Visual observation or visual score	1 = absent 9 = present	1 state	Off-type standard and Uniformity score >5
7DG QN VG/MS	*7G QN VG/MS	31D QN VG/MS	**Root: length**	DUS plot or single plants	At least 400 plants in total from 4 replicates or at least 40 plants in total from	Visual observation or visual score or measurements on single plants	1 = very short 3 = short 5 = medium 7 = long 9 = very long	Clear visual difference or 2 states or COYD @ 5% for both 2 and 3 year tests	Off-type standard and Uniformity score >5 or COYU at 0.1% for both 2 and 3

COYD @5% for both 2 and 3 years tests

Distinctness 5.1

The standard for measured or counted quantitative characters, is, at least, a 5% (P=0.05) significant difference in one character over two or three growing cycles in a Combined Over Years Distinctness (COYD) analysis.

COYD method

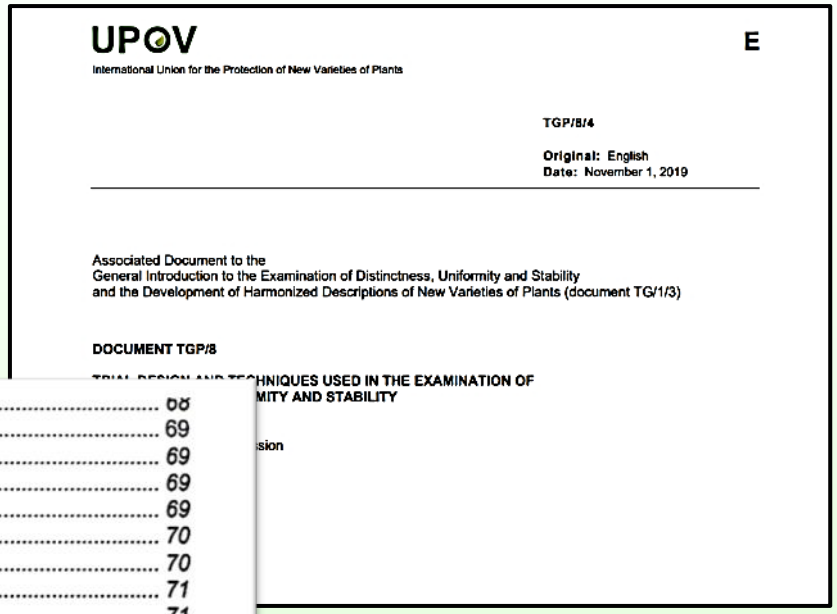
Maize: Method of examination

- **Maize: Method of examination & assessing Distinctness**

		CPVO
Method of Examination	Number of Growing Cycles	two growing cycles
	Test Design	40 plants: inbred lines and single hybrids 60 plants: other hybrids , open-pollinated varieties
	Number of Plants / Parts of Plants to be Examined	Inbred lines and single hybrids: 10 plants or parts taken from each of 10 plants Other types of hybrids: 20 plants or parts taken from each of 20 plants Open-pollinated varieties: 40 plants or parts taken from each of 40 plants
Distinctness	**QN**	visually observed characteristics: at least the span of one note COYD: 1% significance level or less ($p<0.01$) in a test over either two or three years. t-test: 1% significance level or less ($p<0.01$) in two consecutive or two out of three growing cycles.
Uniformity		Inbred lines and single hybrids: population standard of 3% with an acceptance probability of 95%. In the case of a sample of 40 plants, the maximum number of off-types allowed would be 3. three-way cross hybrids, double-cross hybrids and open-pollinated varieties: variability within the variety should not exceed the variability of comparable varieties already known.

COYD method

- **TGP/8 SELECTED TECHNIQUES USED IN DUS EXAMINATION**



UPOV

International Union for the Protection of New Varieties of Plants

E

TGP/8/4

Original: English
Date: November 1, 2019

Associated Document to the
General Introduction to the Examination of Distinctness, Uniformity and Stability
and the Development of Harmonized Descriptions of New Varieties of Plants (document TG/1/3)

DOCUMENT TGP/8

3. The combined over-years criteria for Distinctness(COYD)

COYD method

Combined-Over-Years Distinctness(COYD)

Standard Deviation approach

Requirements for application of COYD method

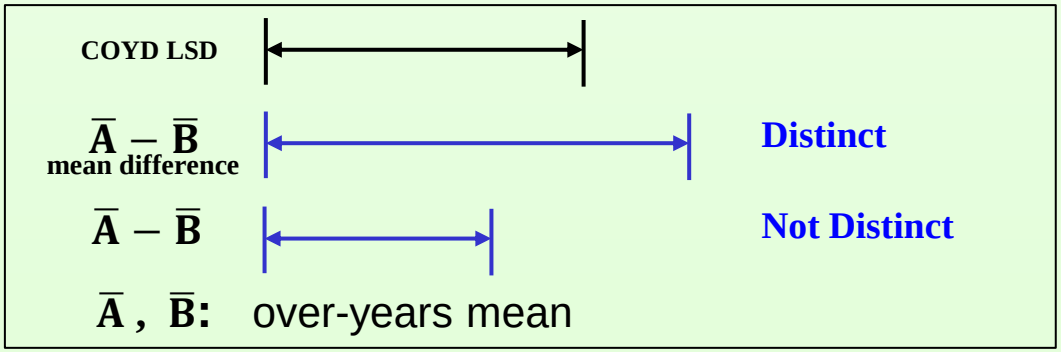
- **Used with data on QN characteristics for cross-pollinated and some self-pollinated varieties**
- **observations are made on a plant basis over at least two years or growing cycles, and at a single location**
- **Need at least 10 df, and preferably at least 20 df for the varieties-by - years mean square**
  - ✓ **This corresponds to 11 comparable varieties, two years of trials or 8 comparable varieties, three years.**
  - ✓ **where there are small numbers of varieties in the test, Long-Term COYD can be used**

COYD method

Combined-Over-Years Distinctness

COYD method

- ✓ **for each characteristic, taking the variety means from the two or three years of trials for candidates and comparable varieties and producing over-year means for the varieties;**
- ✓ **calculate a least significant difference (LSD), based on variety-by-years variation, for comparing variety means;**
- ✓ **if the over-years mean difference between two varieties is greater than or equal to the LSD then the varieties are said to be distinct in respect of that characteristic.**



COYD method

Char.: Days to ear emergence in perennial ryegrass varieties **(14 varieties, 3 years)** *(TGP/8 p.74)*

	Y1	Y2	Y3	mean	Diff. to C2	Result
R1	38	41	35	38	35	D
R2	63	68	61	64	9	D
R3	69	71	64	68	5	D
R4	71	75	67	71	2	
R5	69	78	69	72	1	
R6	74	77	71	74	-1	
R7	76	79	70	75	-2	
R8	75	80	73	76	-3	
R9	78	81	75	78	-5	D
R10	79	80	75	78	-5	D
R11	76	85	79	80	-7	D
C1	52	56	48	52	21	D
C2	72	79	68	73	0	
C3	85	88	85	86	-13	D

1. Get "over-years mean" of varieties

2. Get the smallest difference for "D", COYD LSD = 3.6

3. Get difference over-year mean "C2 - R1", C2 - R1 = 73-38 = 35

4. Compare "C2 - R1 : COYD LSD"

C2 - R1 >COYD LSD Distinct

C2 – R7 < COYD LSD Not Distinct

Mean - year

Targeted candidate variety

COYD LSD = 3.6

COYD method

- **Procedure for COYD**

1. Calculating "over – years mean" of varieties

2. Calculating " LSD" value :

$$LSD_p = t_p \times \sqrt{2} \times SE(\bar{x})$$

$$SE(\bar{x}) = \sqrt{\frac{varieties - by - years\ mean\ squqre}{number\ of\ test\ years}}$$

3. Comparing "*LSD*" to "over – years mean difference between two varieties ($|\bar{x}_1 - \bar{x}_2|$)"

$|\bar{x}_1 - \bar{x}_2| \geq LSD$ ***Distinct***

$|\bar{x}_1 - \bar{x}_2| < LSD$ ***Not Distinct***

COYD method

- **How to get LSD value**

$$LSD_p = t_p \times \sqrt{2} \times SE(\bar{x})$$

t_p ⇒ taken from Student's t table for a two-tailed test with probability *p* and with degree of freedom(df) with the variety-by-years mean square.

$$SE(\bar{x}) = \sqrt{\frac{varieties - by - years\ mean\ squqre}{number\ of\ test\ years}}$$

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Varieties	5883.6	13	452.59	177.91	5.79E-22	2.1192
Years	349.86	2	174.93	68.762	4.15E-11	3.369
Error	66.143	26	2.544			
Total	6299.6	41				

$t_p \quad \sqrt{2} \quad SE(\bar{x})$

$$LSD = 2.779 * 1.414 * \sqrt{\frac{2.544}{3}}$$

$$= 3.6$$

t_p = *tinv (0.01, df)*

= *tinv (0.01, 26)* *(EXCEL)*

=*2.779*

$$SE(\bar{x}) = \sqrt{\frac{2.544}{3}}$$

t Table

cum. prob	$t_{.50}$	$t_{.75}$	$t_{.80}$	$t_{.85}$	$t_{.90}$	$t_{.95}$	$t_{.975}$	$t_{.99}$	$t_{.995}$	$t_{.999}$	$t_{.9995}$
one-tail	0.50	0.25	0.20	0.15	0.10	0.05	0.025	0.01	0.005	0.001	0.0005
two-tails	1.00	0.50	0.40	0.30	0.20	0.10	0.05	0.02	0.01	0.002	0.001
df											
1	0.000	1.000	1.376	1.963	3.078	6.314	12.71	31.82	63.66	318.31	636.62
2	0.000	0.816	1.061	1.386	1.886	2.920	4.303	6.965	9.925	22.327	31.599
3	0.000	0.765	0.978	1.250	1.638	2.353	3.182	4.541	5.841	10.215	12.924
4	0.000	0.741	0.941	1.190	1.533	2.132	2.776	3.747	4.604	7.173	8.610
5	0.000	0.727	0.920	1.156	1.476	2.015	2.571	3.365	4.032	5.893	6.869
6	0.000	0.718	0.906	1.134	1.440	1.943	2.447	3.143	3.707	5.208	5.959
7	0.000	0.711	0.896	1.119	1.415	1.895	2.365	2.998	3.499	4.785	5.408
8	0.000	0.706	0.889	1.108	1.397	1.860	2.306	2.896	3.355	4.501	5.041
9	0.000	0.703	0.883	1.100	1.383	1.833	2.262	2.821	3.250	4.297	4.781
10	0.000	0.700	0.879	1.093	1.372	1.812	2.228	2.764	3.169	4.144	4.587
11	0.000	0.697	0.876	1.088	1.363	1.796	2.201	2.718	3.106	4.025	4.437
12	0.000	0.695	0.873	1.083	1.356	1.782	2.179	2.681	3.055	3.930	4.318
13	0.000	0.694	0.870	1.079	1.350	1.771	2.160	2.650	3.012	3.852	4.221
14	0.000	0.692	0.868	1.076	1.345	1.761	2.145	2.624	2.977	3.787	4.140
15	0.000	0.691	0.866	1.074	1.341	1.753	2.131	2.602	2.947	3.733	4.073
16	0.000	0.690	0.865	1.071	1.337	1.746	2.120	2.583	2.921	3.686	4.015
17	0.000	0.689	0.863	1.069	1.333	1.740	2.110	2.567	2.898	3.646	3.965
18	0.000	0.688	0.862	1.067	1.330	1.734	2.101	2.552	2.878	3.610	3.922
19	0.000	0.688	0.861	1.066	1.328	1.729	2.093	2.539	2.861	3.579	3.883
20	0.000	0.687	0.860	1.064	1.325	1.725	2.086	2.528	2.845	3.552	3.850
21	0.000	0.686	0.859	1.063	1.323	1.721	2.080	2.518	2.831	3.527	3.819
22	0.000	0.686	0.858	1.061	1.321	1.717	2.074	2.508	2.819	3.505	3.792
23	0.000	0.685	0.858	1.060	1.319	1.714	2.069	2.500	2.807	3.485	3.768
24	0.000	0.685	0.857	1.059	1.318	1.711	2.064	2.492	2.797	3.467	3.745
25	0.000	0.684	0.856	1.058	1.316	1.708	2.060	2.485	2.787	3.450	3.725
26	0.000	0.684	0.856	1.058	1.315	1.706	2.056	2.479	2.779	3.435	3.707
27	0.000	0.684	0.855	1.057	1.314	1.703	2.052	2.473	2.771	3.421	3.690
28	0.000	0.683	0.855	1.056	1.313	1.701	2.048	2.467	2.763	3.408	3.674
29	0.000	0.683	0.854	1.055	1.311	1.699	2.045	2.462	2.756	3.396	3.659
30	0.000	0.683	0.854	1.055	1.310	1.697	2.042	2.457	2.750	3.385	3.646
40	0.000	0.681	0.851	1.050	1.303	1.684	2.021	2.423	2.704	3.307	3.551
60	0.000	0.679	0.848	1.045	1.296	1.671	2.000	2.390	2.660	3.232	3.460
80	0.000	0.678	0.846	1.043	1.292	1.664	1.990	2.374	2.639	3.195	3.416
100	0.000	0.677	0.845	1.042	1.290	1.660	1.984	2.364	2.626	3.174	3.390
1000	0.000	0.675	0.842	1.037	1.282	1.646	1.962	2.330	2.581	3.098	3.300
z	0.000	0.674	0.842	1.036	1.282	1.645	1.960	2.326	2.576	3.090	3.291
	0%	50%	60%	70%	80%	90%	95%	98%	99%	99.8%	99.9%
	Confidence Level										

https://towardsdatascience.com/hypothesis-testing-using-t-test-inferential-statistics-part3-6fb43683bc32

COYD method

- **Result of COYD**

TGP/8 p.74

14 varieties, 3 years

	Y1	Y2	Y3	mean	Diff. to C2	Result
R1	38	41	35	38	35	D
R2	63	68	61	64	9	D
R3	69	71	64	68	5	D
R4	71	75	67	71	2	ND
R5	69	78	69	72	1	ND
R6	74	77	71	74	-1	ND
R7	76	79	70	75	-2	ND
R8	75	80	73	76	-3	ND
R9	78	81	75	78	-5	D
R10	79	80	75	78	-5	D
R11	76	85	79	80	-7	D
C1	52	56	48	52	21	D
C2	72	79	68	73	0	
C3	85	88	85	86	-13	D

C2 is Distinct from R1, R2 ,R3, R9, R10,R11

C2 is Not Distinct from R4, R5 ,R6, R7, R8

$|\bar{x}_1 - \bar{x}_2| \geq LSD$ *Distinct*

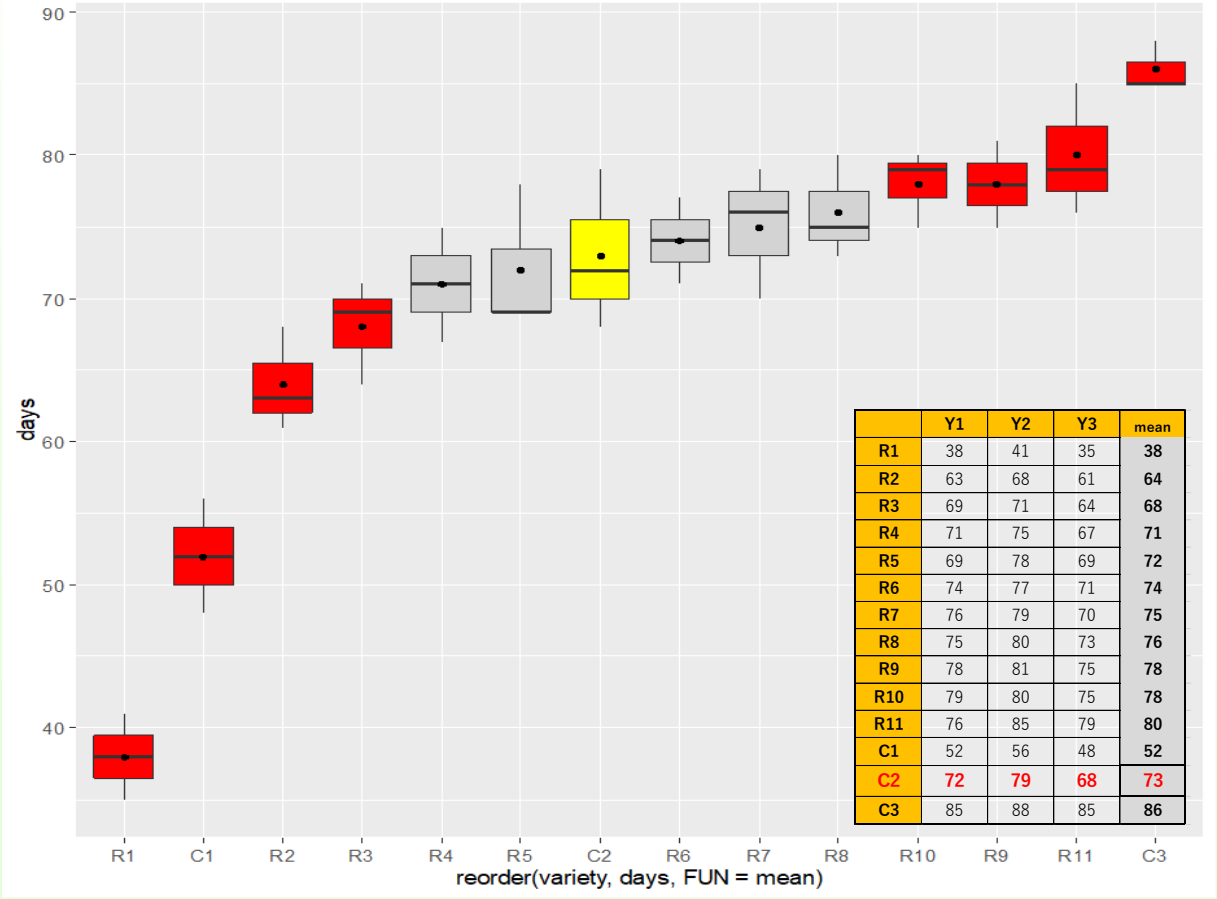
$|\bar{x}_1 - \bar{x}_2| < LSD$ *Not Distinct*

Mean value - year

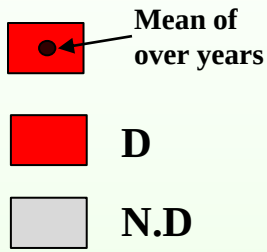
LSD = 3.6

COYD method

- **Result of COYD**

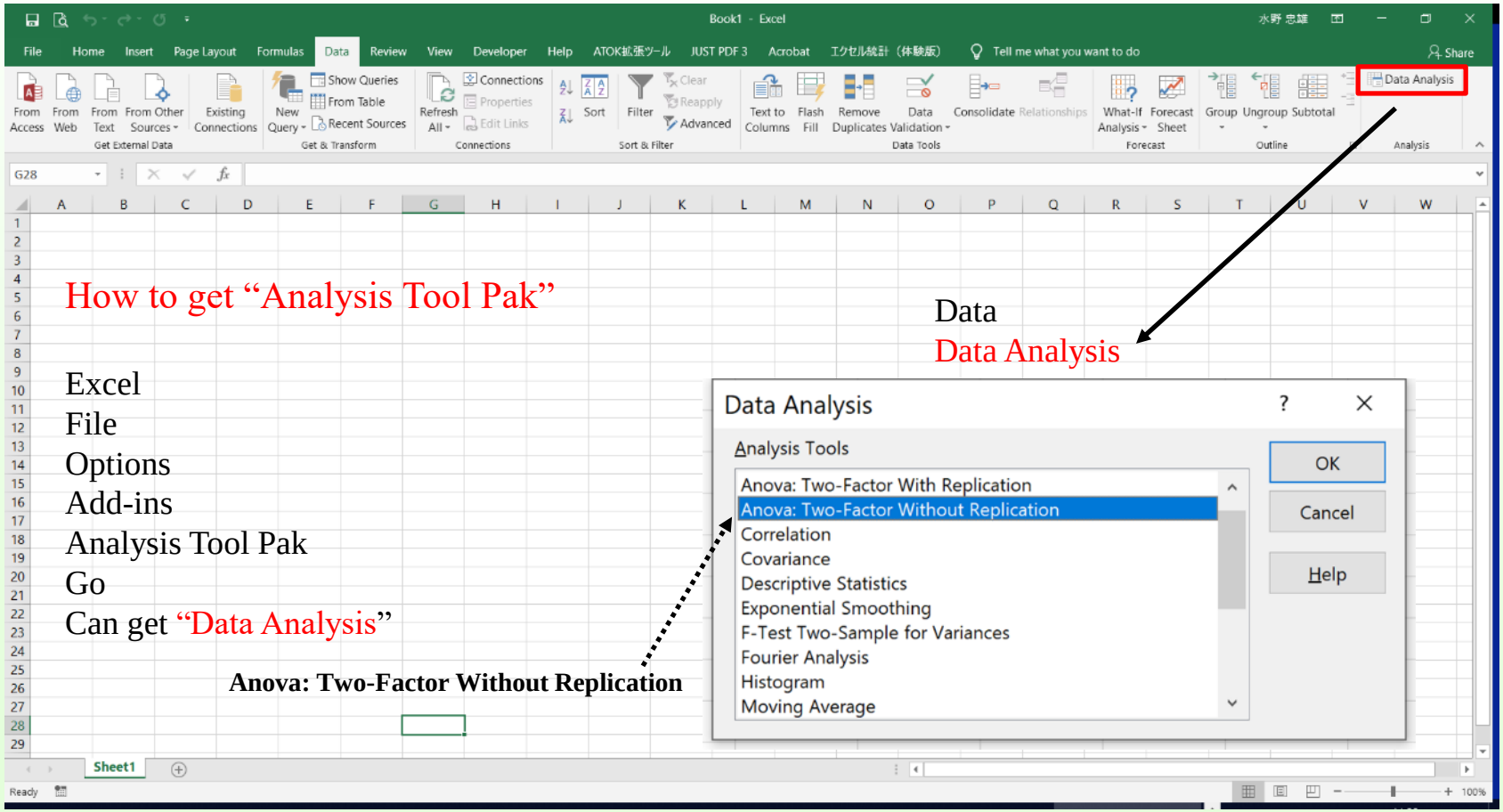


	Y1	Y2	Y3	mean
R1	38	41	35	38
R2	63	68	61	64
R3	69	71	64	68
R4	71	75	67	71
R5	69	78	69	72
R6	74	77	71	74
R7	76	79	70	75
R8	75	80	73	76
R9	78	81	75	78
R10	79	80	75	78
R11	76	85	79	80
C1	52	56	48	52
C2	72	79	68	73
C3	85	88	85	86



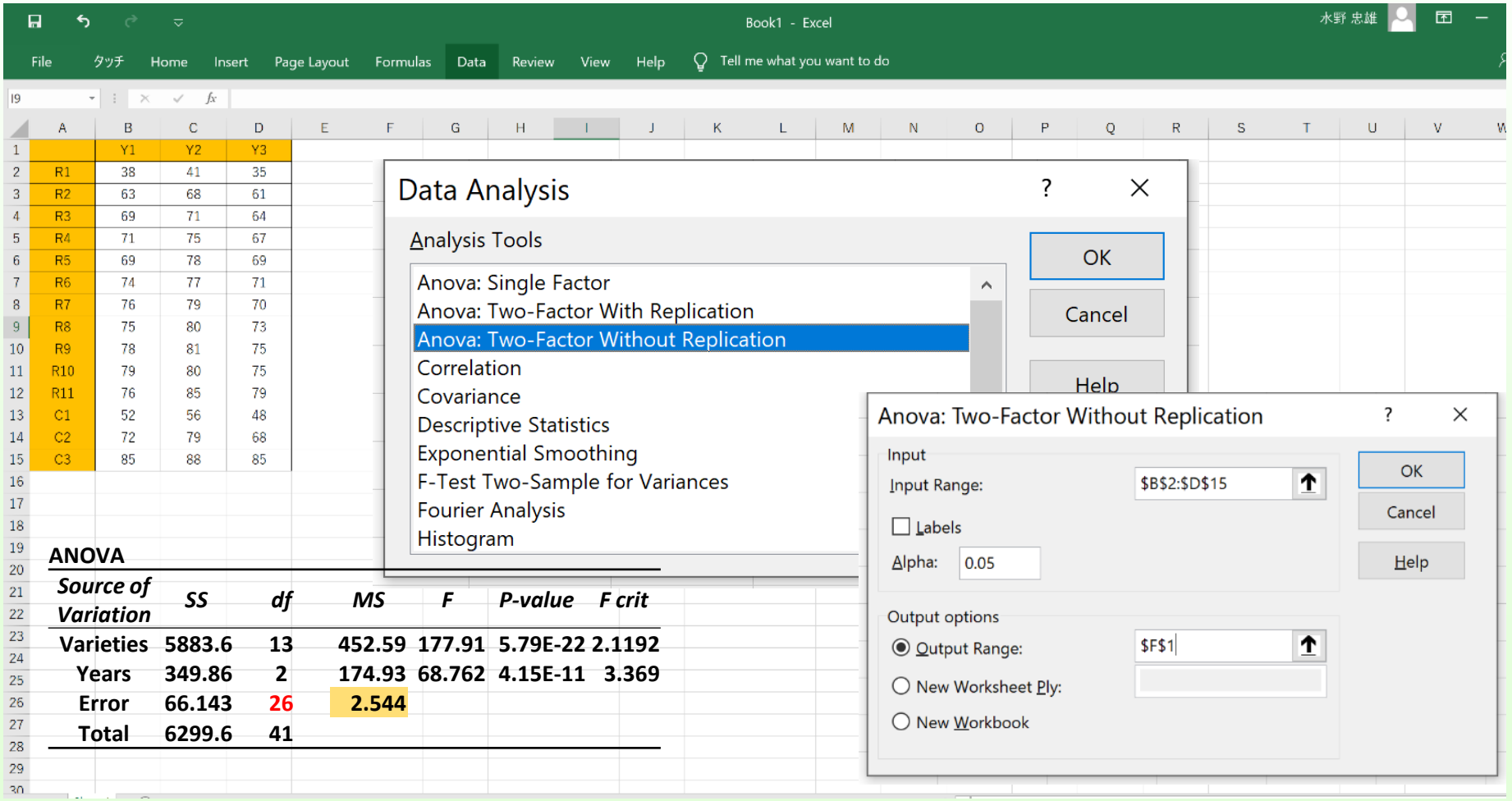
COYD method

- **How to get *varieties by years mean squqre _ ANOVA***



COYD method

- **How to get *varieties – by – years mean squqre _ ANOVA***



	Y1	Y2	Y3
R1	38	41	35
R2	63	68	61
R3	69	71	64
R4	71	75	67
R5	69	78	69
R6	74	77	71
R7	76	79	70
R8	75	80	73
R9	78	81	75
R10	79	80	75
R11	76	85	79
C1	52	56	48
C2	72	79	68
C3	85	88	85

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Varieties	5883.6	13	452.59	177.91	5.79E-22	2.1192
Years	349.86	2	174.93	68.762	4.15E-11	3.369
Error	66.143	26	2.544			
Total	6299.6	41				

U

Cross-pollinated varieties

Uniformity examination

- **QL, PQ => Off-type approach, same as vegetative propagated, self-pollinated varieties**
- **QN => Standard Deviation approach**

COYU method
Relative variance method

Uniformity Examination

Cross-pollinated varieties

■ **Assessment of Uniformity** TGP/10/1 2.5.2

Method of propagation of the variety	QL	PQ	QN
Vegetatively propagated 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 **- Three way cross** **- Double cross**	**Off-types**	**Off-types**	**Standard deviations**

- The most common approaches are listed first.

Uniformity Examination

Cross-pollinated varieties

- **UNITED KINGDOM NATIONAL LIST/ PLANT BREEDERS RIGHTS TECHNICAL PROTOCOL FOR THE OFFICIAL EXAMINATION OF DISTINCTNESS, UNIFORMITY AND STABILITY (DUS)**

CARROT

Character Number										
CPVO TP/ CARROT/1 2004	UPOV TG/206/1 2003	UK	Character	Material examined	Number of plants or sample size for assessment	Method of assessment and recording	States of expression	D Method and Minimum distance required	U Method and Standard applied
1D QN VG	1 QN VG	60D QN VG	**Foliage: width of crown**	DUS plot	At least 400 plants in total from 4 replicates	Visual observation or visual score	3 = narrow 5 = medium 7 = broad	Clear visual difference or 2 states	Off-type standard and Uniformity score >5
2D QN VG	2 QN VG	70D QN VG	**Leaf: attitude**	DUS plot	At least 400 plants in total from 4 replicates	Visual observation or visual score	1 = erect 3 = semi-erect 5 = prostrate	Clear visual difference or 2 states	Off-type standard and Uniformity score >5
3DG QN VG/MS	*3G QN VG/MS	20D QN VG/MS	**Leaf: length (including petiole)**	DUS plot or single plants	At least 400 plants in total from 4 replicates or at least 40 plants in total from 4 replicates	Visual observation or visual score or measurements on single plants	1 = very short 3 = short 5 = medium 7 =long 9 = very long	Clear visual difference or 2 states or COYD @ 5% for both 2 and 3 year tests	Off-type standard and Uniformity score >5 or COYU at 0.1% for both 2 and 3 year tests
4D QN VG	*4 QN VG	45D QN VG	**Leaf: division**	DUS plot	At least 400 plants in total from 4 replicates	Visual observation or visual score	3 = fine 5 = medium 7 = coarse	Clear visual difference or 2 states	Off-type standard and Uniformity score >5
5D QN VG	*5 QN VG	71D QN VG	**Leaf: intensity of green colour**	DUS plot	At least 400 plants in total from 4 replicates	Visual observation or visual score	3 = light 5 = medium 7 = dark	Clear visual difference or 2 states	Off-type standard and Uniformity score >5
6D QL VG	*6 QL VG	24D QL VG	**Leaf: anthocyanin coloration of petiole**	DUS plot	At least 400 plants in total from 4 replicates	Visual observation or visual score	1 = absent 9 = present	1 state	Off-type standard and Uniformity score >5
7DG QN VG/MS	*7G QN VG/MS	31D QN VG/MS	**Root: length**	DUS plot or single plants	At least 400 plants in total from 4 replicates or at least 40 plants in total from	Visual observation or visual score or measurements on single plants	1 = very short 3 = short 5 = medium 7 = long 9 = very long	Clear visual difference or 2 states or COYD @ 5% for both 2 and 3 year tests	Off-type standard and Uniformity score >5 or COYU at 0.1% for both 2 and 3

COYU @0.1% for both 2 and 3 years tests

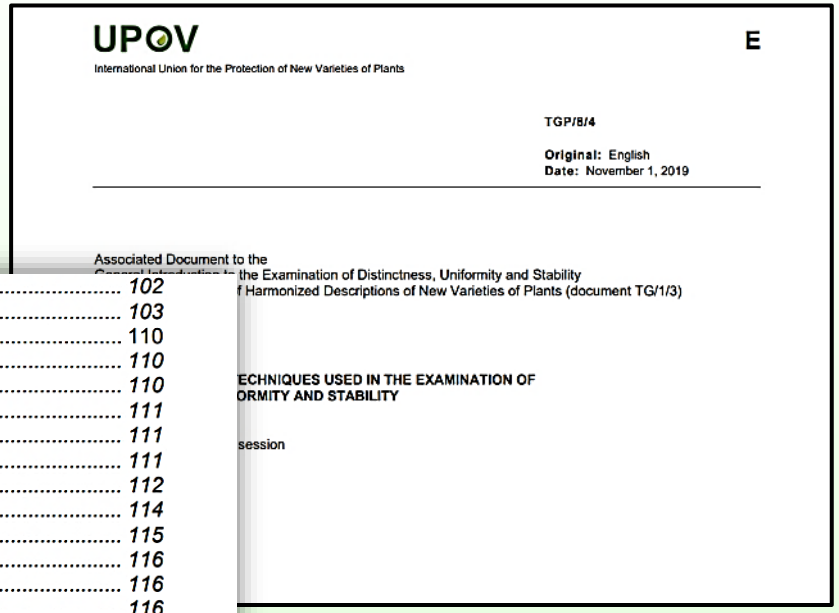
Uniformity

6.4 For cross-pollinated varieties, relative uniformity should be applied

6.10 it can be considered sufficiently uniform after two test cycles if COYU analysis is not significantly greater than that of the reference varieties at the 0.1% (P=0.001) significance level.

COYU method

- **TGP/8 SELECTED TECHNIQUES USED IN DUS EXAMINATION**



UPOV

International Union for the Protection of New Varieties of Plants

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TGP/8/4

Original: English
Date: November 1, 2019

Associated Document to the
the Examination of Distinctness, Uniformity and Stability
f Harmonized Descriptions of New Varieties of Plants (document TG/1/3)

ECHNIQUES USED IN THE EXAMINATION OF
ORMITY AND STABILITY

session

9. The combined over-years uniformity criterion (COYU)

COYU method

Combined Over-Years Uniformity (COYU)

- **COYU method**

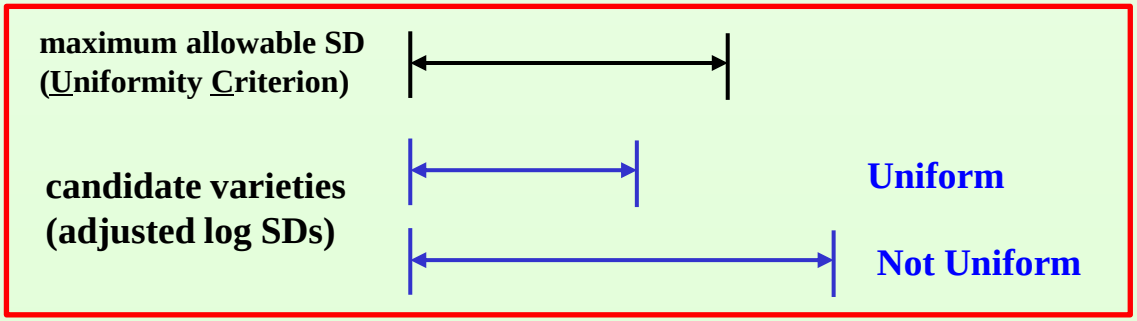
  The COYU method is a method for assessing uniformity using relative tolerances limit calculated based on Standard Deviation (SD) obtained from over several years testing of comparable varieties.

- **Requirements for application of COYU method**

  - **Used with data on measured QN characteristics (MS)**
  - **observations are made on a plant basis over two or more years**
  - **When there are some differences between plants of a variety, representing quantitative variation rather than presence of off-oypes.**
  - **Need at least 20 df**
    - ✓ **This corresponds to 11 comparable varieties, two years of trials or 8 comparable varieties, three years.**

COYU method

- **COYU method**
  1. **Calculation of within-plot SDs for each variety in each year.**
  2. **Transformation of SDs: by adding 1 and converting to natural logarithms.**
  3. **Estimation of the relationship between the SD and mean in each year.**
  4. **Adjustments of log SDs of candidate and comparable varieties based on the estimated relationships between SD and mean in each year.**
  5. **Averaging of adjusted log SDs over years.**
  6. **Calculation of the maximum allowable SD (the Uniformity Criterion).**
  7. **Comparison of the adjusted log SDs of candidate varieties with the UC.**



COYU method

- **Procedure for COYU**

Example data-set : **days to ear emergence: perennial ryegrass** **(12 varieties, 3 years)**

Step: 1. **Calculation of within-plot SDs for each variety in each year.** **(TGP/8, p.115)**

	Character Means			Within Plot SD		
Variety	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
R1	38	41	35	8.5	8.8	9.4
R2	63	68	61	8.1	7.6	6.7
R3	69	71	64	9.9	7.6	5.9
R4	71	75	67	10.2	6.6	6.5
R5	69	78	69	11.2	7.5	5.9
R6	74	77	71	9.8	5.4	7.4
R7	76	79	70	10.7	7.6	4.8
R8	75	80	73	10.9	4.1	5.7
R9	78	81	75	11.6	7.4	9.1
R10	79	80	75	9.4	7.6	8.5
R11	76	85	79	9.2	4.8	7.4
C1	52	56	48	8.2	8.4	8.1

$$SD_j = \sqrt{\frac{\sum_{i=1}^{n}(y_{ij} - y_j)^2}{(n-1)}}$$

$$SD = \frac{\sum_{j=1}^{r} SD_j}{r}$$ (averaged over all replicates)

y_{ij}: measured value

y_j : mean of measured value

n : number of plants

r : number of replicates

COYU method

- **Procedure for COYU**

Step: 2. **Transformation of SDs: by adding 1 and converting to natural logarithms.**

	Character Means			Within Plot SD			Log (SD+1)		
Variety	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
R1	38	41	35	8.5	8.8	9.4	2.25	2.28	2.34
R2	63	68	61	8.1	7.6	6.7	2.21	2.15	2.04
R3	69	71	64	9.9	7.6	5.9	2.39	2.15	1.93
R4	71	75	67	10.2	6.6	6.5	2.42	2.03	2.01
R5	69	78	69	11.2	7.5	5.9	2.50	2.14	1.93
R6	74	77	71	9.8	5.4	7.4	2.38	1.86	2.13
R7	76	79	70	10.7	7.6	4.8	2.46	2.15	1.76
R8	75	80	73	10.9	4.1	5.7	2.48	1.63	1.90
R9	78	81	75	11.6	7.4	9.1	2.53	2.13	2.31
R10	79	80	75	9.4	7.6	8.5	2.34	2.15	2.25
R11	76	85	79	9.2	4.8	7.4	2.32	1.76	2.13
C1	52	56	48	8.2	8.4	8.1	2.22	2.24	2.21

LN(1+8.5) = 2.25 (Excel)

Purpose of transformation：
is to make the SDs easier to handle in statistical analysis.

COYU method

- **Procedure for COYU**

Step:

3. **Estimation of the relationship between the SD and mean in each year. The method used is based on moving averages of the log SDs of comparable varieties ordered by their means.**
4. Adjustments of log SDs of candidate and comparable varieties **based on the estimated relationships between SD and mean in each year.**
5. **Averaging of adjusted log SDs over years.**

Adjust

	Character Means			Within Plot SD			Log (SD+1)			Over-Year Means		Adj. Log (SD+1)		
Variety	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3	Char. mean	Adj. Log (SD+1)	Year 1	Year 2	Year 3
R1	38	41	35	8.5	8.8	9.4	2.25	2.28	2.34	38	2.26	2.36	2.13	2.3
R2	63	68	61	8.1	7.6	6.7	2.21	2.15	2.04	64	2.1	2.32	2	2
R3	69	71	64	9.9	7.6	5.9	2.39	2.15	1.93	68	2.16	2.42	2.1	1.95
R4	71	75	67	10.2	6.6	6.5	2.42	2.03	2.01	71	2.15	2.43	1.96	2.06
R5	69	78	69	11.2	7.5	5.9	2.50	2.14	1.93	72	2.2	2.52	2.14	1.96
R6	74	77	71	9.8	5.4	7.4	2.38	1.86	2.13	74	2.12	2.36	1.84	2.16
R7	76	79	70	10.7	7.6	4.8	2.46	2.15	1.76	75	2.14	2.43	2.19	1.8
R8	75	80	73	10.9	4.1	5.7	2.48	1.63	1.90	76	2.02	2.44	1.7	1.91
R9	78	81	75	11.6	7.4	9.1	2.53	2.13	2.31	78	2.3	2.52	2.16	2.24
R10	79	80	75	9.4	7.6	8.5	2.34	2.15	2.25	78	2.22	2.33	2.23	2.09
R11	76	85	79	9.2	4.8	7.4	2.32	1.76	2.13	80	2.01	2.28	1.78	1.96
Mean										70	2.15	2.4	2.02	2.04
C1	52	56	48	8.2	8.4	8.1	2.22	2.24	2.21	52	2.19	2.32	2.08	2.17

COYU method

Procedure for COYU

Step:

6. **Calculation of the maximum allowable SD (the Uniformity Criterion: UC).**

7. **Comparison of the adjusted log SDs of candidate varieties with the maximum allowable SD.**

Variety	Over-Year Means		Adj. Log (SD+1)		
	Char. mean	Adj. Log (SD+1)	Year 1	Year 2	Year 3
R1	38	2.26	2.36	2.13	2.3
R2	64	2.1	2.32	2	2
R3	68	2.16	2.42	2.1	1.95
R4	71	2.15	2.43	1.96	2.06
R5	72	2.2	2.52	2.14	1.96
R6	74	2.12	2.36	1.84	2.16
R7	75	2.14	2.43	2.19	1.8
R8	76	2.02	2.44	1.7	1.91
R9	78	2.3	2.52	2.16	2.24
R10	78	2.22	2.33	2.23	2.09
R11	80	2.01	2.28	1.78	1.96
Mean	70	2.15	2.4	2.02	2.04
C1	52	2.19	2.32	2.08	2.17

$$UC_p = SD_r + t_p\sqrt{V\left(\frac{1}{k}+\frac{1}{Rk}\right)}$$

SD: ***2.15;*** Over-Year mean of Adj. Log(SD+1)

t_p : ***3.118;*** Student's t table with p=0.002 (one-tailed) and 30 degrees of freedom

tinv(0.002*2,30) = 3.118 (Excel)

V: ***0.0202;*** variance of the adjusted log SDs after removing year effects ; MS of Varieties within years in ANOVA

k: ***3;*** number of years

R: ***11;*** number of comparable varieties

$$UC_p = SD_r + t_p\sqrt{V\left(\frac{1}{k}+\frac{1}{Rk}\right)} = 2.15 + 3.118x\sqrt{0.0202x\left(\frac{1}{3}+\frac{1}{3x11}\right)} = 2.42$$

mean adjusted log (SD + 1) ≤ 2.42 Uniform

COYU method

- How to get *Mean Square of Varieties within years*

Variety	Over-Year Means		Adj. Log (SD+1)		
	Char. mean	Adj. Log (SD+1)	Year 1	Year 2	Year 3
R1	38	2.26	2.36	2.13	2.3
R2	64	2.1	2.32	2	2
R3	68	2.16	2.42	2.1	1.95
R4	71	2.15	2.43	1.96	2.06
R5	72	2.2	2.52	2.14	1.96
R6	74	2.12	2.36	1.84	2.16
R7	75	2.14	2.43	2.19	1.8
R8	76	2.02	2.44	1.7	1.91
R9	78	2.3	2.52	2.16	2.24
R10	78	2.22	2.33	2.23	2.09
R11	80	2.01	2.28	1.78	1.96
Mean	70	2.15	2.4	2.02	2.04
C1	52	2.19	2.32	2.08	2.17

Analysis of variance table for adjusted log (SD+1)

Source of variation	Df	Sum Sq	Mean Sq
Year	2	1.01069	0.50535
Varieties within years(=residual)	30	0.60527	0.02018
Total	32		

V: ***0.0202;*** variance of the adjusted log SDs after removing year effects ; MS of Varieties within years in ANOVA

Df :

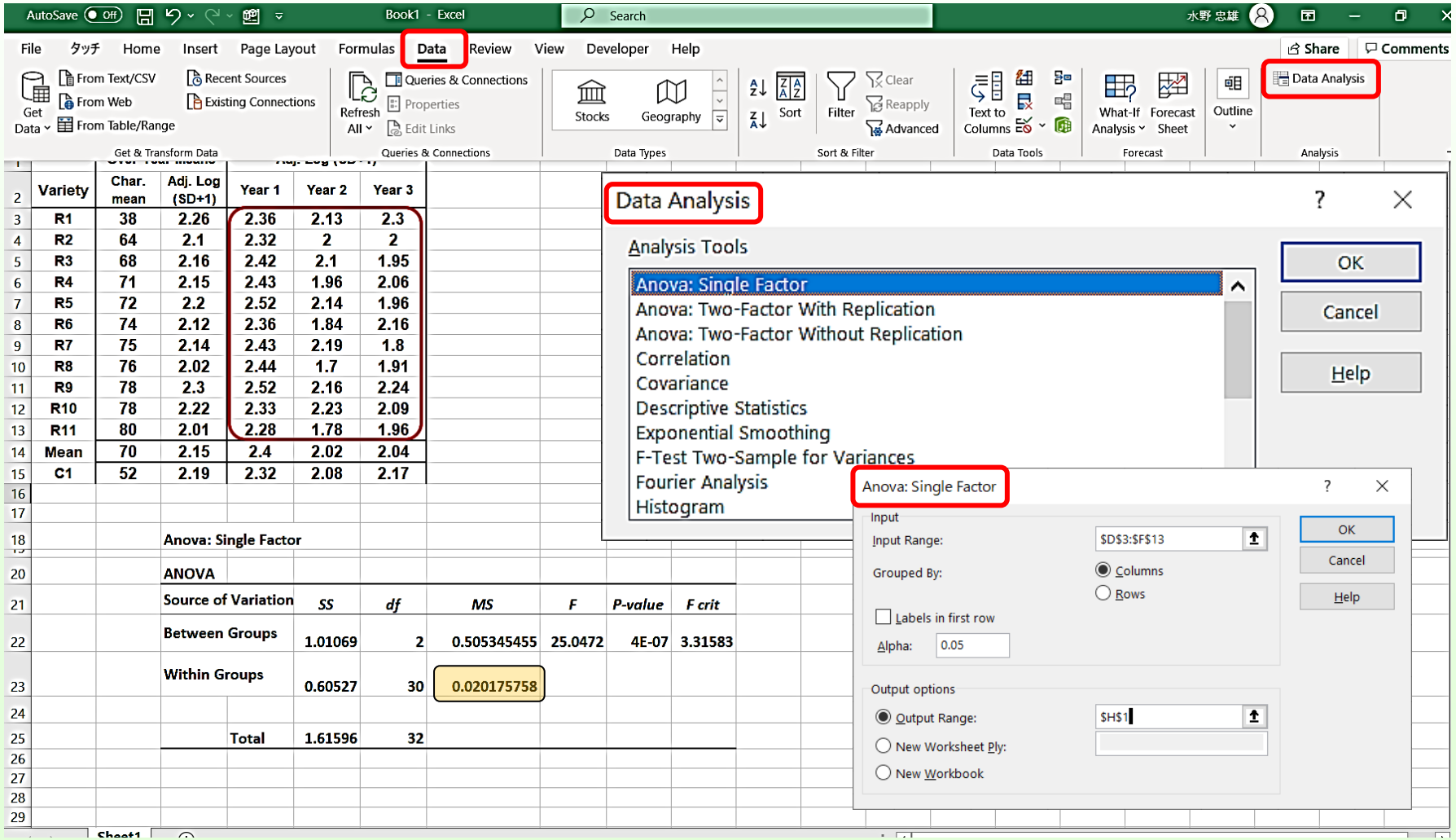
Year: (number of year – 1) =2

Varieties within year = (number of Varieties – 1) (number of years)

= 30

COYU method

- How to get *Mean Square of Varieties within years*



Variety	Char. mean	Adj. Log (SD+1)	Year 1	Year 2	Year 3
R1	38	2.26	2.36	2.13	2.3
R2	64	2.1	2.32	2	2
R3	68	2.16	2.42	2.1	1.95
R4	71	2.15	2.43	1.96	2.06
R5	72	2.2	2.52	2.14	1.96
R6	74	2.12	2.36	1.84	2.16
R7	75	2.14	2.43	2.19	1.8
R8	76	2.02	2.44	1.7	1.91
R9	78	2.3	2.52	2.16	2.24
R10	78	2.22	2.33	2.23	2.09
R11	80	2.01	2.28	1.78	1.96
Mean	70	2.15	2.4	2.02	2.04
C1	52	2.19	2.32	2.08	2.17

Anova: Single Factor

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.01069	2	0.505345455	25.0472	4E-07	3.31583
Within Groups	0.60527	30	0.020175758			
Total	1.61596	32				

Relative variance method

- **TGP/8 SELECTED TECHNIQUES USED IN DUS EXAMINATION**

10. Uniformity assessment on the basis of the relative variance method

UPOV

International Union for the Protection of New Varieties of Plants

E

TGP/8/4

Original: English
Date: November 1, 2019

Associated Document to the
General Introduction to the Examination of Distinctness, Uniformity and Stability
and the Development of Harmonized Descriptions of New Varieties of Plants (document TG/1/3)

USED IN THE EXAMINATION OF
STABILITY

Relative Variance Method

- **Relative variance method**

  - ✓ applied to any measured characteristic that is a continuous variable, irrespective of the method of propagation of the variety.
  - ✓ In cross-pollinated varieties, taking 60 plants for measurements per characteristic per variety.
  - ✓ Calculation of relative variance
    - ➢ Relative variance = $\frac{\textit{variance of the candidate}}{\textit{average variance of the comparable varieties}}$
  - ✓ Compare Relative variance with Thresholds of F Table of the relevant sample size

Relative variance ≤ threshold	**Uniform**
Relative variance > threshold	**Not Uniform**

Relative variance method

Relative variance method

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

average variance =5.32

- ✓ 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: threshold 1.05 < 1.47, Relative variance is less than threshold
- ✓ therefore, **the candidate variety is uniform for that characteristic**

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

Relative variance method

Threshold limit for relative variance for some different sample sizes

TGP/8 p.124

Sample size of candidate	Threshold limit for relative variance (S^2)
30	1.70
40	1.59
50	1.53
60	1.47
80	1.41
100	1.36
150	1.29
200	1.25

Usage of COYD and COYU

COYU Standards **TWC/21/7**

Herbage Crops:

- Festuca, June grass, Lotus corniculatus L., Lucerne, Ryegrass, Tall vat.grass, Timothy, Red Clover, White Clover, …..

Other Agricultural Crops:

- Beta vulgaris L., Hordeum vulgare L., Lupins, Oilseed Rape, Turnip, Rape, Vicia faba L., …..

Vegetables and Other Crops:

- Beetroot, Leaf Beet, Black Radish, Cabbage, Brussels Sprouts, Sprouting Broccoli, Calabrese, Carrot, Celery, Onion, Shallot, Leek, Parsnip, Sweet melon, Turnip, Watermelon, ….

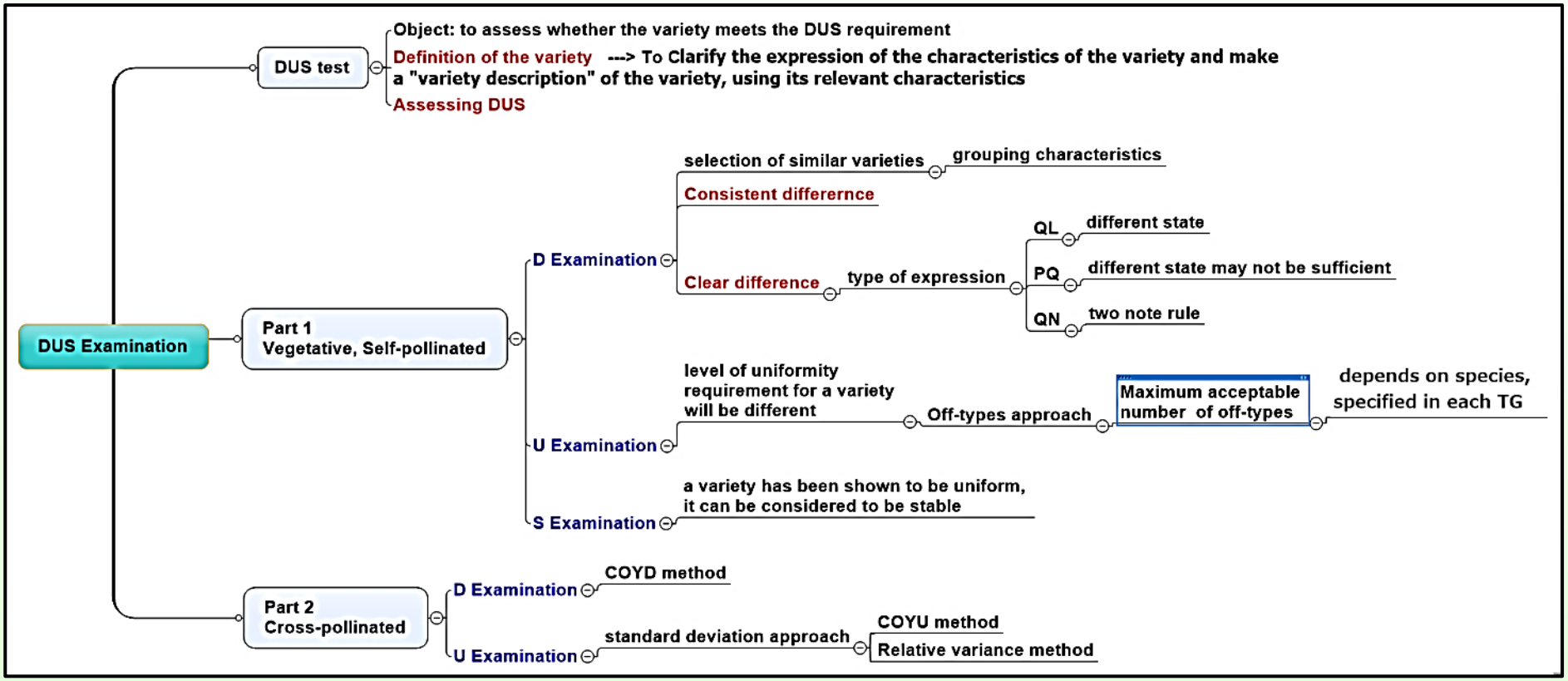
Types of Characteristics by Crop

Number of types of characteristics:

	QL	PQ	QN	Total
Rice	10	20	35	65
Tomato	10	3	33	46
Maize	2	3	36	41
Carrot	4	6	22	32

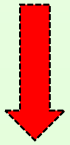
How to assess the QN characteristics is the point of DUS examination !!

Summary


DUS Examination
DUS test
Object: to assess whether the variety meets the DUS requirement
Definition of the variety ---> To Clarify the expression of the characteristics of the variety and make a "variety description" of the variety, using its relevant characteristics
Assessing DUS
Part 1
Vegetative, Self-pollinated
D Examination
selection of similar varieties
grouping characteristics
Consistent differernce
Clear difference
type of expression
QL
different state
PQ
different state may not be sufficient
QN
two note rule
U Examination
level of uniformity requirement for a variety will be different
Off-types approach
Maximum acceptable number of off-types
depends on species, specified in each TG
S Examination
a variety has been shown to be uniform, it can be considered to be stable
Part 2
Cross-pollinated
D Examination
COYD method
U Examination
standard deviation approach
COYU method
Relative variance method

Thank you for your attention

Any Question ? Please feel free to email me !!



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