



TGP/9.3.2Draft1

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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

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/9

“EXAMINING DISTINCTNESS”

Section TGP/9.3.2: The Use of ‘Phenotypic Distance’ for Examining Distinctness

Document prepared by expert from France

to be considered by the

Technical Working Party on Automation and Computer Programs (TWC), at its twentieth session to be held in Texcoco, Mexico, from June 17 to 20, 2002

Technical Working Party for Vegetables (TWV), at its thirty-sixth session to be held in Tsukuba, Japan, from September 9 to 13, 2002

Technical Working Party for Agricultural Crops (TWA), at its thirty-first session to be held in Rio de Janeiro, Brazil, from September 23 to 27, 2002

Technical Working Party for Ornamental Plants and Forest Trees (TWO), at its thirty-fifth session to be held in Quito, from November 18 to 22, 2002

Technical Working Party for Fruit Crops (TWF), at its thirty-third session to be held in San Carlos de Bariloche, Argentina, from November 25 to 29, 2002

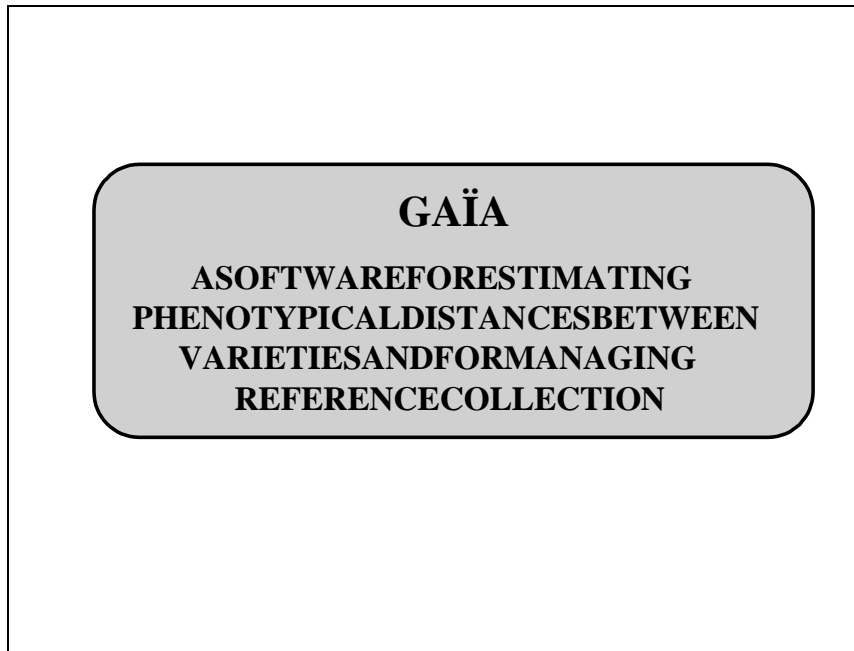
SECTION 9.3.2
THE USE OF 'PHENOTYPIC DISTANCE' FOR A MINING DISTINCTNESS

1. This contribution has been planned to be a part of the document TGP/9, "Examining Distinctness." In practice, it is closely linked to document TGP/4, "Management of Variety Collections" which considers the ways to identify the varieties of common knowledge to which a candidate variety must be compared in the examination of distinctness. ety
2. With this objective, France developed a system which is described in the Annex to this document. The main aims are the following ones:
 - (a) having large variety collections for some species, the limitation of the number of varieties which must be grown with the candidate varieties;
 - (b) the improvement of the grouping system based on a characteristic by characteristic basis as recommended by UPOV, which is not always efficient enough;
 - (c) the simulation of what the examiner does when comparing two varieties: the combination of differences on a set of characteristics, each of them being not necessarily sufficient to clearly distinguish the varieties.
3. In order to have a secure system, the notion of "Distinctness Plus" has been introduced. It means that, based on a computation of the difference taking into account their size and the reliability of each characteristic, the threshold used to sort out a variety is larger than the minimum distance used by the expert to establish distinctness. With this approach, it becomes possible to develop a software and to get a good automation of this system for the application.
4. For each species, this system must be calibrated to determine the weight which can be given to each difference and to evaluate the reliability of each characteristic in a given environment and for the genetic variability concerned. It means that the role of the expert remains essential.
5. As examples some figures are presented based on applications on Maize and Oilseed Rape in France (see the two last pages of the Annex).
6. A portable version of the software is under preparation and it should be available in 2003 to all UPOV members.

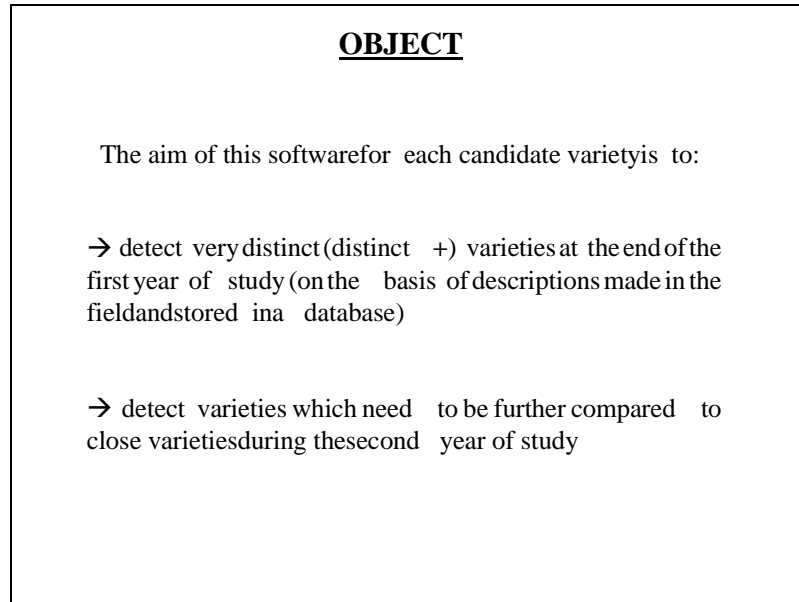
[Annex follows]

ANNEX

Slide1



Slide2



Slide3

PRINCIPLE

→ The estimation of the phenotypical distance between 2 varieties is based on the addition of the differences observed for the different characteristics

→ Each difference observed is weighted by the crop expert according to the value of the difference and to the reliability of each characteristic

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Maize example

	Type of grain (QL)	Attitude of blade (QL)	Attitude of lateral branches (QL)	Anthocyanin coloration of glumes (QL)	Length of husks (QL)	Number of rows of grain (QL)	Diameter of the ear (QT)	Time of anthesis (QT)
Variety A	4	3	7	2	5	3	3	6
Variety B	4	3	5	1	7	3	5	7
Difference	0	0	2	1	2	0	2	1
Weight	0	0	2	0	0	0	2	0

$\Sigma W=4$

Estimation of the phenotypical distance between A and B

(QL)=qualitative characteristics
(QT)=quantitative characteristics

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RULESFORDECISION

→Ifthe phenotypicaldistance between A and B \geq threshold
(fixed bythe crop expert)

A and Bare declared distinct +and arenot directly
comparedinthesecond year

→Ifthe phenotypicaldistance between A and B < threshold

A and B willbedirectlycomparedside by side in
the field inthesecond year

Slide6

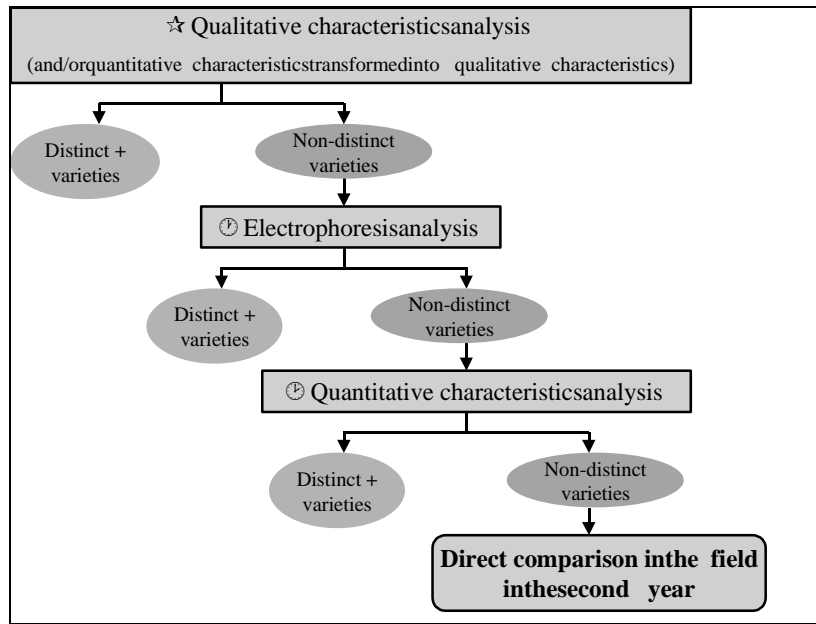
**DIFFERENTTYPESOFCHARACTERISTICSCAN
BEUSEDINTHESOFTWARE**

→Qualitative characteristics observed ina1to9 scale
or transformedinto 1to 9 scale

→Quantitative characteristics measured

→ Electrophoreticcharacteristics observed as presence orabsence
of eachallele

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☆- **QUALITATIVE ANALYSIS**

	Type of grain (QL)	Attitude of blade (QL)	Attitude of lateral branches (QL)	Anthocyanin coloration of glumes (QL)	Length of husks (QL)	Number of rows of grain (QL)	Diameter of the ear (QF)	Time of anthesis (QL)
Variety A	4	3	7	2	5	3	3	6
Variety B	4	3	5	1	7	3	5	7
Difference	0	0	2	1	2	0	2	1
Weight	0	0	2	0	0	0	2	0

$D_1=4$

		Variety A								
		1	2	3	4	5	6	7	8	9
Variety B	1	0	0	2	3	3	3	3	3	3
	2		0	0	2	3	3	3	3	3
	3			0	0	2	3	3	3	3
	4				0	0	2	3	3	3
	5					0	0	2	3	3
	6						0	0	2	3
	7							0	0	2
	8								0	0
	9									0

For each characteristic, necessity to define the matrix of weights

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Result of the step ☆(qualitative analysis)

Maize :The threshold for distinctness is 6

→ If $D_1 \geq 6$ A and B are distinct +

→ In this case, $D_1 < 6$ A and B are not distinct +
we go to step ⌚ (electrophoresis analysis)

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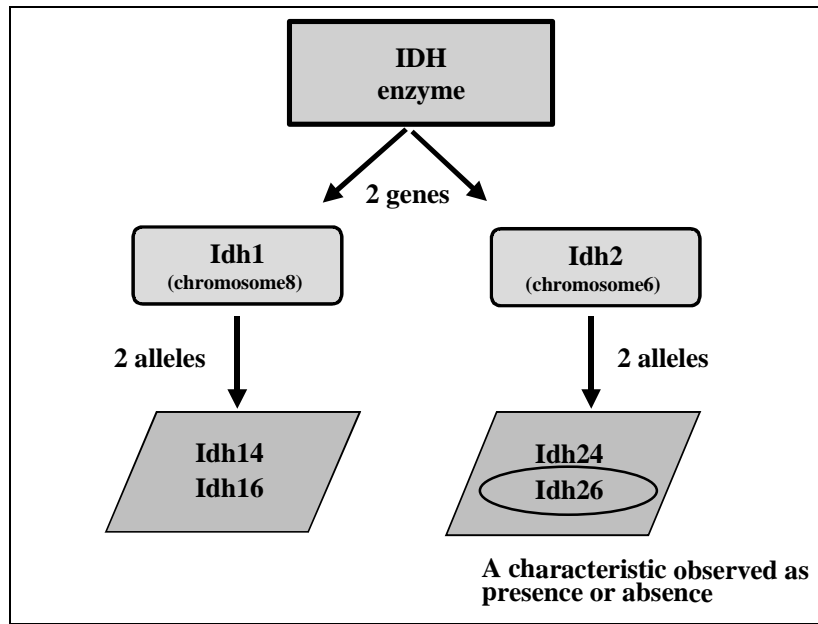
⌚- **ELECTROPHORESIS ANALYSIS**

→ 10 enzymes are used for maize

- malate dehydrogenase (MDH)
- isocitrate dehydrogenase (IDH)
- 6-phosphogluconate dehydrogenase (PGD)
- phosphoglucomutase (PGM)
- phosphoglucoisomerase (PGI)
- acid phosphatase (ACP)
- diaphorase (DIA)
- alcohol dehydrogenase (ADH)
- glutamate oxalo-transaminase (GOT)
- catalase (CAT)

→ electrophoretic characteristic = homozygote allele

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Maizeexample

	Chromosome8		Chromosome6	
	Idh14	Idh16	Idh24	Idh26
VarietyA	0	1	1	0
VarietyB	0	1	0	1
Difference	0	0	1	1

Number of differences

Number of chromosomes on which differences are observed

$$\text{Distance} = 2 \times 0,25 + 1 \times 1 = 1,5$$

Weight associated to the number of differences

Weight associated to chromosomes

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Rules for decisionatstep (electrophoresis analysis)

→ It is not possible to establish distinctness solely on the basis of a difference found in a characteristic derived by using electrophoresis

→ In maize, necessity to have a phenotypical distance based on qualitative characteristics ≥ 2 to take into account the electrophoresis results

→ After step and:

- 2 varieties A and B are distinct + if $D_1 + D_2 \geq 6$
with $D_1 \geq 2$

- in our example, A and B are not distinct + ($D_1 + D_2 = 5,5$)
we go to step

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QUANTITATIVE ANALYSIS

Rule of distinctness: a weight is associated when 2 differences are observed in 2 trials (1 trial = 1 location + 1 year)

Maize example: Length of plant

	Trial1	Trial2
Variety A	176cm	190cm
Variety B	140cm	152cm
Difference	36cm	38cm
Weight	3	6

Trial1 20% \Rightarrow 37cm
15% \Rightarrow 28cm

Trial2 20% \Rightarrow 32cm
15% \Rightarrow 24cm

2 threshold values are fixed by the crop expert

- If difference between A and B $< 15\%$ of the mean length of the trial \Rightarrow weight = 0

- If difference between A and B $\geq 15\%$ of the mean length of the trial \Rightarrow weight = 3

- If difference between A and B $\geq 20\%$ of the mean length of the trial \Rightarrow weight = 6

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Rules for decision of the step (quantitative analysis)

→ The crop expert for maize have decided to choose the lowest of the 2 weights (in this case 3)

$D_3=3$

→ Summary :

$D_1=4,2$ (-contribution of quantitative characteristic transformed into qualitative characteristics)
 $D_2=1,5$
 $D_3=3$

} $\Sigma D=6,5$

A and B are distinct +

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Results obtained in 2000

2420 inbred lines in the reference collection
307 new inbred lines in the first year of study

GAIA

83682 comparisons to be done

- 142 candidate varieties are distinct + (43 without electrophoresis)
- 165 candidate varieties are not distinct +

A candidate variety has on average 5,25 non distinct varieties (17,7 without electrophoresis)

==> 864 comparisons must be done in the field in the second year of study

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OTHER USES OF GAÏA IN FRANCE

→ Officially :for rapeseed

→ Undertest:for cerealsandsunflower

☞ ☞ ☞ ☞ ☞ ☞

RAPESEED

→ GAÏA is applied on lines which are treated as mainly self-pollinated varieties

→ 14 qualitative characteristics, 2 quantitative characteristics and 7 electrophoretic characteristics

→ As for maize, 2 trials are made each year in 2 locations (La Minière, Le Magneraud)

→ The phenotypical distance is calculated by taking into account the two locations : for each characteristic, the weights are given if differences are observed in the two locations with the same sense

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Results obtained in 2000/2001

255 inbred lines in the reference collection
63 new inbred lines candidate

↙ ↘

GAÏA

19971 comparisons to be done

- 3 candidate varieties are distinct +
- 60 candidate varieties are not distinct +:

A candidate variety has on average 17 non-distinct varieties

- 31 varieties have between 1 and 10 non-distinct varieties
- 8 varieties have between 11 and 20 non-distinct varieties
- 8 varieties have between 21 and 30 non-distinct varieties
- 8 varieties have between 31 and 40 non-distinct varieties
- 5 varieties have more than 41 non-distinct varieties

==> 1040 comparisons must be done in the field in the second year of study

[End of Annex and of document]