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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

TECHNICAL WORKING PARTY FOR AGRICULTURAL CROPS

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SOFTWARE USING PHENOTYPIC DISTANCE FOR DISTINCTNESS

Document prepared by experts from France

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GAÏA

A SOFTWARE FOR ESTIMATING PHENOTYPICAL DISTANCES BETWEEN VARIETIES AND FOR MANAGING REFERENCE COLLECTION

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OBJECT

The aim of this software for each candidate variety is to :

➠ detect very distinct (distinct +) varieties at the end of the first year of study (on the basis of descriptions made in the field and stored in a database)

➠ detect varieties which need to be further compared to close varieties during the second year of study

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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	

Σ W= 4

Estimation of the phenotypical distance between A and B

(QL) = qualitative characteristics

(QT) = quantitative characteristics

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RULES FOR DECISION

➠ If the phenotypical distance between A and B ≥ threshold (fixed by the crop expert)

A and B are declared distinct + and are not directly compared in the second year

➠ If the phenotypical distance between A and B < threshold

A and B will be directly compared side by side in the field in the second year

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DIFFERENT TYPES OF CHARACTERISTICS CAN BE USED IN THE SOFTWARE

➠ Qualitative characteristics	observed in a 1 to 9 scale or transformed into 1 to 9 scale
➠ Quantitative characteristics	measured
➠ Electrophoretic characteristics	observed as presence or absence of each allele

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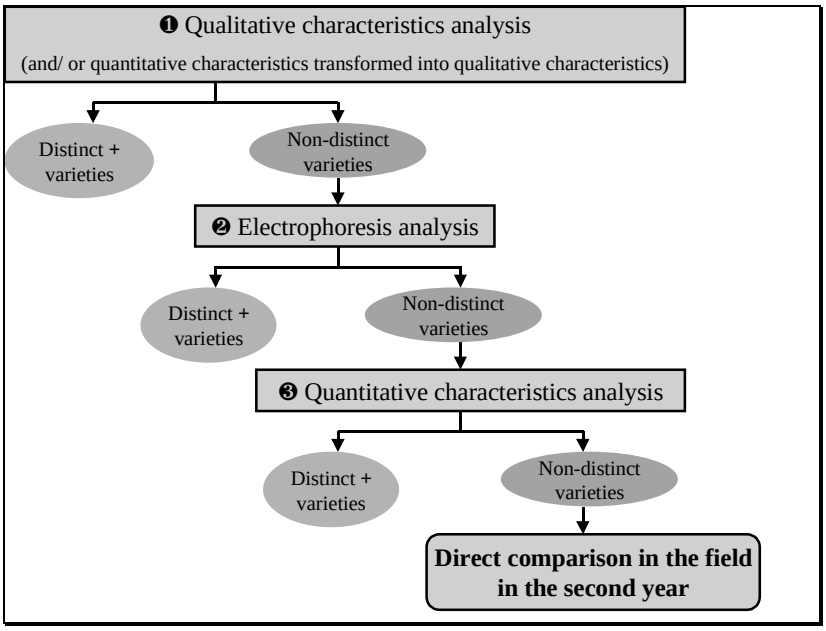

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❶ - QUALITATIVE ANALYSIS

	Type of grain (QL)	Atitude 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 (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 B \ Variety A	1	2	3	4	5	6	7	8	9
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 ❶ (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 ❷ (electrophoresis analysis)

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❷ - 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 deshydrogenase (ADH)
- glutamate oxalotransaminase (GOT)
- catalase (CAT)

➠ electrophoretic charateristic = homozygote allele

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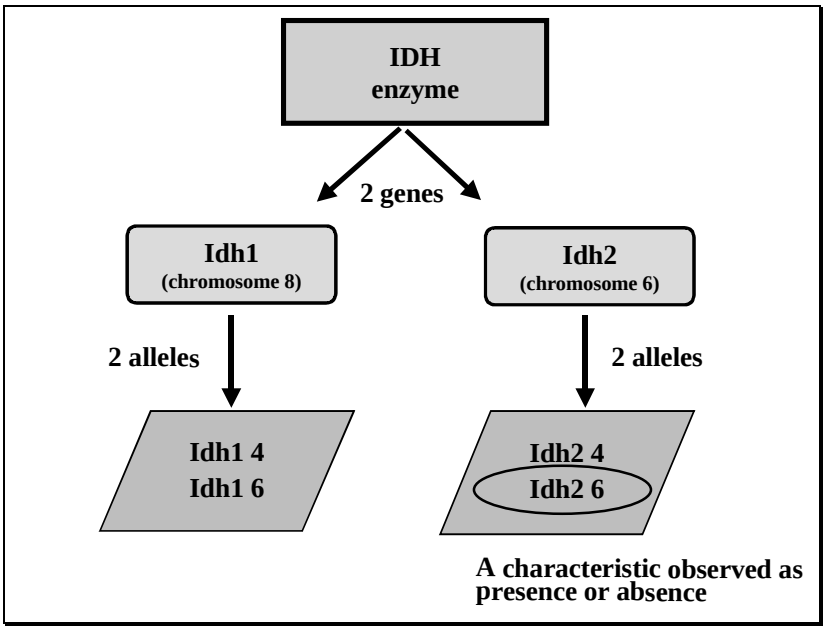

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Maize example

	Chromosome 8		Chromosome 6	
	Idh1 4	Idh1 6	Idh2 4	Idh2 6
Variety A	**0**	**1**	**1**	**0**
Variety B	**0**	**1**	**0**	**1**
Difference	**0**	**0**	**1**	**1**

Number of differences

Number of chromosomes on which differences are observed

Distance 2 = 2 x 0,25 + 1 x 1 = 1,5

Weight associated to the number of differences

Weight associated to chromosomes

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Rules for decision at step ❷ (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 ❶ and ❷ :

- 2 varieties A and B are distinct + if $\mathbf{D_1 + D_2 \geq 6}$ with $\mathbf{D_1 \geq 2}$
- in our exemple, A and B are not distinct + ($\mathbf{D_1 + D_2 = 5,5}$)
  we go to step ❸

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❸ - 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

	Trial 1	Trial 2
Variety A	176 cm	190 cm
Variety B	140 cm	152 cm
Difference	36 cm	38 cm
Weight	**3**	**6**

Trial 1	20%	37 cm
	15%	28 cm
Trial 2	20%	32 cm
	15%	24 cm

2 threshold values are fixed by the crop expert

- If difference between A and B < 15% of the mean lenght of the trial — weight = 0
- If difference between A and B ≥ 15% of the mean lenght of the trial — weight = 3
- If difference between A and B ≥ 20% of the mean lenght of the trial — weight = 6

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Rules for decision of the step ❸ (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 characteristics 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

836 882 comparisons to be done

GAÏA

- 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
- Under test : for cereals and sunflower

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 sens

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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

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