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INTERNATIONALUNIONFORTHEPROTECTIONOFNEWVARIETIESOFPLANTS GENEVA

TECHNICALWORKINGPA RTY ON AUTOMATIONANDCOMPU TERPROGRAMS

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BANDSCORING

Document prepared by experts from France and the United Kingdom

1. <u>ScoringofMolecularData</u>

1. For a number of years protein electrophoresis has been recognized as a valuable tool in identifying and discriminating between crop varieties. Agr eed methods are part of the UPOV TestGuidelinesforalimited number of crops. Much research has taken place over recent years demonstrating the potential of DNA approaches. It is the purpose of this section to outline issues that need to be addressed hendealing with DNA data.

- 2. TherearemanyDNAprofilingmethodsavailabletocropscientists.
 - AFLP
 - SSR
 - (andvariantsoftheabove)
 - Sequencedata

3. It is important to select scoring strategies that reflect the genetics of the crop under study (e.g. ploidy) and the mode of operation of the DNA profiling system (e.g. a co -dominant system). For example, the number of samples that are needed will depend on the observed (or

assumed) level of within variety uniformity. For self -pollinated varieties, clonal material and inbred lines, a limited number of samples may be required to have a sample representative of such varieties. For allogamous crops large samples sizes are required with individuals making upthepopulation generating band frequencies.

4. BeforestartingtoscoreDNAprofilingsystemsanumberofissuesneedtobeaddressed. Someofthesearegivenbelowandwilldependontheend -useoftheresults.

- Aremonomorphicbandstobescoredoronlypolymorphicones?
- Include"faint"bands?
- Howarerareallelestobehandled?
- Arethedatatobeaddedtoadatabase?

5. Oncethesepointshavebeenagreedthenstandardprotocolsneedtobedrawnuptoensure consistencyofscoringinthelong -termirrespectiveo fthepersonnelinvolved.

6. Whenscoringisdonemanually, it may be necessary to have two independents corings and include only those identified by both scorers. This will become more important when assessments are made across -gels.

7. Where 'automated scoring' is applied, the band intensity threshold, or other user tunable parameters, needs to be researched and fixed, as far as possible, to increase the robustness of the scoring system (re -scoring the same gels on another occasion or in different laboratories using the same 'system'). With high -intensity multiplexed systems, manual scoring may be difficult.

8. Whileinsomecircumstancesmissingdatacanbeaccommodated in the calculation of the distance estimator, there will be occasions where a consistent treatment of missing data will be required. Removing an individual from the subsequent analysis just because it has a small fraction of missing data, is adrastic step. There are an umber of proposed strategies for dealing with modestamounts of missing data. (See papert obepublished by Law and Van Eeuwijk).

9. For data required for databasing, the choice of markers is as important as the quality assurance applied to any generated data. Markers of choic e may not always be those with optimal PIC values, but will depend much more on the ease of scoring, "robustness", freely available, sufficiently polymorphic and other factors such as multiplexibility.

2. <u>DistanceMeasures(basedonapaperbyH.P.Piep hoandF.Laidig)</u>

10. Based on either banding data or allelic data, distances and similarity measures can be computed.Suchmeasuresmaybeviewedasconvenientmeansofdatareduction,andtheyneed notinvolveany genetic concept(Weir, 1990: 163). Some of the measures for allelic data were designed based on genetic models specifying the processes underlying the divergence of populations.Itshouldbecheckedwhetherornotthese assumptions are metinpractice.

2.1 Binarybandingdata

SneathandSokal(1973)listfourclassesofsimilaritymeasures: (i)distancecoefficients,
(ii) association coefficients, (iii) correlation coefficients, and (iv) probabilistic similarity
coefficients.Mostmeasuresrelevantfortheanalysiso
fbinarybandingdatafallwithintheclass

of association measures, which are based on qualitative data (multi -state or two -state). Occasionally association measures turn out to be special cases of distance coefficients or correlation coefficients. For a comprehensive overview, see Sneath and Sokal (1973) and CliffordandStephenson(1975).Inthefollowing, we will give a few measures which we found to be frequently used in genetical studies. The data of two genotypes can be arranged as $2x^2$ frequency table

			x
		0	1
	0	<i>n</i> ₀₀	<i>n</i> ₀₁
У	1	n_{10}	n_{11}

12. From this basic table the following frequencies can be computed (Armstrong et al., undated)

 n_{00} =numberofbandpositionsscored0for xand0for v n_{10} =numberofbandpositionsscored 1for *x*and0for v n_{01} =numberofbandpositionsscored0for xand1for y $n_{11} = n_{xy}$ = number of bandpositions scored 1 for xand1for v $n_x = n_{01} + n_{11} =$ numberofbandspresentin х $n_v = n_{10} + n_{11}$ =numberofbandspresentin v $n_{xy} = n_{00} + n_{11} =$ num berofmatches $n = n_{00} + n_{10} + n_{01} + n_{11}$ = number of band positions

13. The most important distinction is between measures that ignore negative matches (0, 0 comparisons) and measures that do not. It is debatable whether or not exclusion of neg matchesisuseful in the context of DNA profiles. Take the following simple example with three genotypes x, y and z:

Table3:Exampleofscoresforbandingdataoftwogenotypes *x*, *y* and *z* (4bandpositions):

Bandposition		1234
Genotype	x y z	0011 1111 0111

14. In a way, z is as similar to x as it is to y, because in both comparisons, three of four comparisons are concordant. The only difference is that in the z-x comparison, only two of the three concordant observations are positive matches, while in the z-y comparisons all are positive matches. On the other hand, for a negative match to be observable, the corresponding band must be observed for at least one of the other genotypes. Thus, any similarity measure which takes into account negative matches, will depend on the particular set of genotypes included in the study, which is a point infavor of measure signoring negative matches. Moreover, there are

several ways in which a genotype may lose a band/DNA fragment, so it may be argued that basing similarity on the mutual absence of a character is inappropriate (Vierling and Nguyen, 1992).

15. The similarity measures (s) given here take values in the range from zero to unity. For identical genotypes s=1, while for completely distinct measures s=0. The distance measure corresponding to the sessimilarity measures may be computed as 1 - s.

2.1.1 Measuresthatignorenegativematches

(1) NeiandLi(1979):

 $NL_{xy}=2 n_{xy}/(n_x+n_y)=2 n_{11}/(2n_{11}+n_{01}+n_{10})$

16. This is probably the most popular similarity measure in genetic analyses. It is equivalent to the Dice coefficient (Sneath and Sokal, 1973: 131) and assesses the proportion of bands shared by two genotypes x and y. Under certain statistical assumptions, NL_{xy} may be employed to derive an estimate of the mean number of nucleotide substitutions per nucleotide site (Nei and Li, 1979), which is a useful parameter in evolutionary studies. The underlying assumptions are probably realistic in natural population s, but doubt ful in plant breeding. If the computations are exclusively based on single -banded RFLP patterns, then NL_{xy} is equal to Rogers distance (see below) (Melchinger, 1993).

(2) Jaccard(SneathandSokal,1973:131):

 $J_{xy} = n_{11}/(n - n_{00}) = n_{11}/(n_{01} + n_{10} + n_{11})$

17. NL_{xy} is the same as J_{xy} , except that positive matches carry double weight. It has been suggested (Link et al ., 1995) that NL_{xy} is more appropriate for RFLP data, while J_{xy} shouldbe used with RAPD data. The reasoning is as follows (Engqvist and Becker, 1995): RAPD markers either produce a band in a certain position or the band is absent. Thus, one band position usually corresponds to one marker locus. By contrast, RFLPs produce fragments of varyinglengthsfordifferentalleles. Fortwocultivarsdiffering at a marker locus, fragments are produced for both alleles, but they differ in their position on the gel. Hence a locus is represented by two band positions. When the cultivars are identical, however, the locus is manifestin only one band position for the pairwise comparison. Thus, matches should receive NL_{xy}. This reasoning implies that RAPDs show double weight compared to mismatches, as in nolengthpolymorphismsandthateachRFLPalleleproducesonlyonebandonthegel. Bothof these assumptions are idealizations, but may be reasonable approximation in practice.

2.1.2 Measures, which treat positive and negative matches a like

18. These measures are symmetric in n_{00} and n_{11} , i.e. the formula stays the same when n_{00} and n_{11} are exchanged. Only the most popular measure is given here. For other measures, see Sneathand Sokal (1973) and Clifford and Stephenson (1975).

(3) SimpleMatching(SneathandSokal(1973:132)

 $SM_{xy} = m_{xy}/n = (n_{11} + n_{00})/n$

19. The simple matching coefficient measures the proportion of positive and negative matches. In order to compare SM_{xy} with measures that ignore negative matches, we computed some similarities for the example in Table 3. SM_{xy} yields the same similarity/distance for the pairs x-z and y-z, while measures ignoring negative matches such as J_{xy} and NL_{xy} indicate a larger similarity between y and z.

2.2 Allelicfrequencydataandbandfrequencydata

20. In the following x_i and y_i will denote the frequencies of allele *i* at a given locus for genotypes *x* and *y*, respectively. Alternatively, x_i and y_i maydenote the band frequency at band position *i*, when banding data are used.

(1) Euclideandistance

21. The frequencies x_i and y_i can be viewed as coordinates of points in a multidimensional space. The geometric distance may be interpreted as distance between populations x and y.

 $E_{xy} = [\sum_{i} (x_i - y_i)^2]^{0.5}$

22. When all elic data from sever all ociare available, the distances for individual locimay be averaged. E_{xy} takes a value between 0 and $\sqrt{2}$. A standardization to values between 0 and 1 leads to

(2) Rogers'distance(Nei,1987:211)

 $RD_{xy} = [0.5 \ \sum_{i} (x_i - y_i)^2]^{0.5}$

23. In the i mportant case that x and y are inbred lines and allelic data are used, Rogers' distance RD_{xy}) equals the percentage of lociwhich differ between lines x and y. Its expectation is related to the coefficient of coancestry (Melchinger *etal*., 1991). The R ogers distance has the following deficiency: When the two populations are both polymorphic but share no common alleles, this measure can be come much smaller than unity even if the populations have entirely different sets of alleles (Nei, 1987:209).

(3) Nei'sstandardgeneticdistance

24. Nei'smeasureisintendedforallelicdata.Whennoallelicinformationisavailable,itmay becomputedfrombandfrequencydata.Ifthisisdone,however,themeasuredoesnothavethe genetic interpretation as if computed from allelic data. The *normalized identity of genes* or simply *geneticidentity* isgivenby

 $I_{XY} = J_{XY} / (J_X J_Y)^{1/2}$

25. Where $j_{xy} = \sum x_i y_i$, $j_x = \sum x_i^2$, $j_y = \sum y_i^2$ and J_x , J_y and J_{xy} are the averages of j_x , j_y and j_{xy} over all scored loci. I_{xy} is 1 when the two populations have identical gene frequencies over all loci and is 0 when the yshare no alleles. Because of this property, I_{xy} has been used for measuring

the extent of genetic similarity between populations. The quantity D_{xy} = -Ln(I_{xy}) is the standard genetic distance. Under the assumption that the rate of gene substitution per locus is uniform across both loci and lineages and some other assumptions, it is an estimator for the number of codon differences per locus between two populations x and y (Nei, 1987: 218; Nei, 1972). While I_{xy} ranges from zero to unity, D_{xy} varies between zero and infinity.

Loarce *et al*. (1996) computed the genetic identity based on band frequencies of RAPD fragments from bulked DNA samples of two rye cultivars. O'Donoughue *et al*. (1994) computed D_{xy} forband frequencies from RFLPs inoats. When computed from band frequency data, D_{xy} probably does not allow the interpretation as a measure of the number of codon differences, though the interpretation as a measure.

26. SeeexamplesinAppendixI.

3. DistinctnessandUniformity

27. UPOVhasnotyetestablishedhowbiomoleculardatacouldbeusedintheprocessofDUS testing.DiscussionsareongoinginBMTandothergroup s.DuringthelastsessionsofBMT, a numberofpapersondifferentcropswerepresentedwiththeaimofexploringhowbiomolecular datacouldhelptodescribeanddistinguishvarieties, and alsotocheckhowuniform the varieties are.

28. The d iscriminant power of biomolecular data is great. On the one hand, there has been some concern about this discriminating power being too big, or more appropriate to assess essential derivation than to assess DUS. On the other hand, the phenotypic traits a re often susceptible to the effect of environment, while it should not be the case for biomolecular data if the method is carefully selected and technically well controlled.

29. Some concern about varieties not being uniform for biomolecular dat a has also been expressed. Averylargemajorityofthepaperssubmitted to the BMT indicated that varieties do not have the same level of uniformity according to their reproduction system, as is the case for phenotypic traits; but within plant, and with in variety uniformity can be compared to what is found using phenotypic traits.

30. The uncontrolled use of biomolecular techniques could open the gate to the use of hundreds of new "characteristics". This is not acceptable in the UPOV context w here the strength of the protection right is essential. UPOV is considering how the use of biomolecular techniques might be used in a way which does not undermine the value of protection (for instance, setthe principles of use, select which methods would be appropriate and reproducible and describe how the data should be used in the scope of DUS testing).

31. The data already provided through the various sessions of the BMT have shown that biomoleculardatahaveapotentialinterestandmightb etakenintoaccountwhentheprinciples and modalities of their use can be established. There are different types of data available, different ways to compare or summarize them, and UPOV will have to establish how the data will be used in the DUS decisio n process to ensure harmonization between countries.

4. Databasesforvarietyinformation

32. The storage of the information related to the varieties examined is very often kept in computerfiles, although some information and notes are kept on different papers as well.

33. In common language, the existence of information in a computer file is incorrectly referred to as "the data is kept in a data base" . The storage of information in a spread sheet, for instance Excel \bigcirc , where lines ar e different varieties (or lots) and columns are information in order to identify the variety (i.e. variety code) and information obtained (i.e. allele found) is such an example. This is very convenient when the number of lines and columns is small as it allows a lot of functions (sort, average,...) on the data and nice outputs in order to provide descriptions or reports.

34. When the amount of information is increasing, and when it is necessary to provide simultaneous access to many users, adataba seis needed. Access to the data is then controlled by software which allows different levels of access to the data (right to read, create, update, delete).

35. When a database is needed the data has to be analyzed in order to provide models to describe them and their relationships . A model can comprise several tables, in the most simple cases, to more than a hundred "tables" . Each table is the equivalent of a row x column spreadsheet, where the rows are the different elements of information, and the columns the different types of information needed to identify the object, and describe it. Database design and implementation is usually performed by computer experts in cooperation with the users of the information. Databased esign is neasytask, and usually adesign has a costand so an economical value, which is why designs are sometimes protected, and rarely "given on request".

36. Administrative and technical description of the varieties already known, and understudy, is commonly stored indatabases by the offices incharge of DUS testing and the issue of titles of protection.

37. The notes which are used to provide the description are sometimes in a database and sometimes directly in a provide the description are sometimes in a database and sometimes directly in a sometime of the description are sometimes in a database and sometimes directly in a sometime of the description are sometimes in a database and sometimes directly in a sometime of the description are sometimes in a database and sometimes directly in a sometime of the description are sometimes in a database and sometimes directly in a sometime of the description are sometimes in a database and sometimes directly in a sometime of the description are sometimes in a database and sometimes directly in a sometime of the description are sometimes in a database and sometimes directly in a sometime of the description are sometimes in a database and sometimes directly in a sometime of the description are sometimes in a database and sometimes directly in a sometime of the description are sometimes are sometimes in a database and sometimes directly in a sometime of the description are sometimes directly in a sometime of the description are sometimes are sometimes are sometimes and sometimes directly in a sometime of the description are sometimes are sometimes are sometimes and sometimes directly in a sometimes directly directly

38. The results from biomolecular studies are sometimes in databases, sometimes in special files. F or the time being (except for electrophoresis), as biomolecular results are not used in routine DUS tests, it is not necessary to include th e results in the official administrative and technical databases.

39. InternationalorganizationssuchasUPOVortheEuropeanUnion,forinstance,developed
databases in which information on known varieties and varieties under study are availabl
e. In
thispaper,onlytheUPOVapproachisdescribed.

40. In 1996, UPOV *ad hoc* subgroups proposed a format description for providing variety information. Since then, a UPOV ROM has been produced by UPOV and sent to members. The UPOV ROM is al so available on subscription. The format is described in the Circular U 246209/11/1996 in which 30 -40 types of information are described and identified by TAGs. The description of the varieties is not yet included, but this is not due to computer restri ctions. Plant variety denominations was one of the reasons to develop this database. UPOV provides

on the CD $\,$ -ROM the database itself, the data, a program to search the database, and other documents of interesting dfformat.

Theoptionchos enbyUPOVistheuseofSGML.SGMLisanabbreviationfor"Standard 41. Generalized Markup Language" which was first defined by an International Standard in 1986 In this system the information is provided in electronic text files and the recognition of the informationismadebytheuseoftags . Forinstance, in the UPOVROM the tag < 600 > is used to indicate that the following information in the file is the breeder's reference of the variety under treatment. In the general scope of SGML, XML "Extensible M arkup Language" is the referencefornewdevelopmentsforexchangeofinformation(descriptionoftheinformationand exchange), while HTML "Hyper Text Markup Language", is used to describe the display of information on the computer screens through the int ernet. Many software packages provide basic tools to create or input XML files, whilst, with the present SGML UPOV format, each organization must write and maintain its own specific software to cope with the required specifications.

42. Todefin eadatabasesub -modelforthepurposeofinformationexchange, anotheroptionis possible. Insuchacase, the information can be provided without the use of tags, as the content and specifications are known by the definition of the database model . This approach is probably better if the aim is to reach harmonization and encourage cooperative work, as it allows easier exchange, and the possibility to interconnect different databases at real time .

43. Examples of how information can be kept in different ways is illustrated in Appendix 2.

44. In both cases, (Markup language or database sub -model) when information is exchanged and grouped, the identification of the objects is essential in order to be able to search for information comin gfrom different sources, or tore late objects (varieties for instance) studied in different places. UPOV has already defined such identification codes for the characteristics in the UPOV Test Guidelines . By contrast, the variety code is given by each co untry, and no databaselink is provided in the UPOV ROM to identify if a given variety has been described by one or several countries . The use of species name can help to identify such cases, but this is left to the use of the database . The coding of the species is not yet harmonized by UPOV although some work has been done in that direction.

45. Itisprematuretoimaginehowbio -molecularresults would be input in an administrative and technical database, as these results are not yet used by U POV members for DUS . Nevertheless, two possible uses can be imagined in the future. The first one has already been used for electrophorosis, where UPOV defined adhoc characteristics in the guidelines for the corresponding species. With this approach, t here is no difference in essence with the way agronomic characteristics are used. A variety has a value or note for a characteristic. The second one is already used for agronomic characteristics where different values are obtained beforeafinal"note"i sgiven. In this case, for each variety there is a set of values (i.e. . measure on 60 individual plants from 3 different trials) . We can already imagine in such a case that at least the lot, the sample, the material used, the test, the locus, the allele, the frequency, will enableustocopewithmost, if not all, of the situations where we will have to compute criteria fordistinctnessanduniformity.

APPENDIXI

46. Examples of 3 similarity measures, each on the same dataset, and with the add it is not of 6 monomorphic bands, follow:

CorrelationBetweenOriginal'Raw'Data(10bands)&+Mono(10+6monomorphicbands)

NL	0.9444
J	0.9496
RT	0.9990

CorrelationBetweenSimilarityMethods'Raw'Data

	NL J	
J	0.9843	
RT	0.8349 0	.8609
	NL-M J-N	Λ
J-M	NL-M J-N 0.9968	Λ
J-M RT-M		

NL:-Nei&Li	
J:-Jaccard's	
RT:-RogerandTanimoto	
NL-M(Nei&Libasedon'Raw'+6Monomorphicsbands)	
J-M(Jaccard'sbasedon'Raw'+6Monomorphicsbands)	
RT-M(RogersandTanimotobasedon'Raw'+6Monomo	rphicsbands)

NEI and LI (=DICE)

(MONO BANDS ADDED)

	0.06	0.12	0.17	0.33	0.36	0.30	0.33	0.36	0.39
	7	5	6	3	8	0	3	4	1
0.06		0.05	0.11	0.26	0.30	0.23	0.27	0.30	0.33
7		9	1	3	0	8	3	4	3
0.12	0.05		0.05	0.20	0.23	0.18	0.21	0.25	0.28
5	9		3	0	8	2	7	0	0
0.17	0.11	0.05		0.14	0.18	0.13	0.16	0.20	0.23
6	1	3		3	2	0	7	0	1
0.33	0.26	0.20	0.14		0.04	0.08	0.12	0.15	0.18
3	3	0	3		3	3	0	4	5
0.36	0.30	0.23	0.18	0.04		0.04	0.07	0.11	0.14
8	0	8	2	3		0	7	1	3
0.30	0.23	0.18	0.13	0.08	0.04		0.03	0.07	0.10
0	8	2	0	3	0		7	1	3
0.33	0.27	0.21	0.16	0.12	0.07	0.03		0.03	0.06
3	3	7	7	0	7	7		4	7
0.36	0.30	0.25	0.20	0.15	0.11	0.07	0.03		0.03
4	4	0	0	4	1	1	4		2
0.39	0.33	0.28	0.23	0.18	0.14	0.10	0.06	0.03	
1	3	0	1	5	3	3	7	2	

JACCARDS

(MONO BANDS ADDED)

	0.12	0.22	0.30	0.50	0.53	0.46	0.50	0.53	0.56
	5	2	0	0	8	2	0	3	3
0.12		0.11	0.20	0.41	0.46	0.38	0.42	0.46	0.50
5		1	0	7	2	5	9	7	0
0.22	0.11		0.10	0.33	0.38	0.30	0.35	0.40	0.43
2	1		0	3	5	8	7	0	8
0.30	0.20	0.10		0.25	0.30	0.23	0.28	0.33	0.37
0	0	0		0	8	1	6	3	5
0.50	0.41	0.33	0.25		0.08	0.15	0.21	0.26	0.31
0	7	3			3		4	7	3
0.53	0.46	0.38	0.30	0.08		0.07	0.14	0.20	0.25
8	2	5	8	3		7		0	0
0.46	0.38	0.30	0.23	0.15	0.07		0.07	0.13	0.18
2	5	8	1	4	7		1	3	8
0.50	0.42	0.35	0.28	0.21	0.14	0.07		0.06	0.12
0	9	7	6	4	3	1		7	5
0.53	0.46	0.40	0.33	0.26	0.20	0.13	0.06		0.06
3	7	0	3	7	0	3	7		3
0.56	0.50	0.43	0.37	0.31	0.25	0.18	0.12	0.06	
3	0	8	5	3	0	8	5	3	

ROGERS and TANIMOTO

(MONO BANDS ADDED)

	0.11	0.22	0.31	0.54	0.60	0.54	0.60	0.66	0.72
	8	2	6	5	9	5	9	7	0
0.11		0.11	0.22	0.47	0.54	0.47	0.54	0.60	0.66
8		8	2	6	5	6	5	9	7
0.22	0.11		0.11	0.40	0.47	0.40	0.47	0.54	0.60
2	8		8	0	6	0	6	5	9
0.31	0.22	0.11		0.31	0.40	0.31	0.40	0.47	0.54
6	2	8		6	0	6	0	6	5
0.54	0.47	0.40	0.31		0.11	0.22	0.31	0.40	0.47
5	6	0	6		8	2	6	0	6
0.60	0.54	0.47	0.40	0.11		0.11	0.22	0.31	0.40
9	5	6	0	8		8	2	6	0
0.54	0.47	0.40	0.31	0.22	0.11		0.11	0.22	0.31
5	6	0	6	2	8		8	2	6
0.60	0.54	0.47	0.40	0.31	0.22	0.11		0.11	0.22
9	5	6	0	6	2	8		8	2
0.66	0.60	0.54	0.47	0.40	0.31	0.22	0.11		0.11
7	9	5	6	0	6	2	8		8
0.72	0.66	0.60	0.54	0.47	0.40	0.31	0.22	0.11	
0	7	9	5	6	0	6	2	8	

NEI and LI (=DICE)

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	0.33	0.50	0.60	1.00	1.00	0.75	0.77	0.80	0.81
	3	0	0	0	0	0	8	0	8
0.33		0.20	0.33	0.71	0.75	0.55	0.60	0.63	0.66
3		0	3	4	0	6	0	6	7
0.50	0.20		0.14	0.50	0.55	0.40	0.45	0.50	0.53
0	0		3	0	6	0	5	0	8
0.60	0.33	0.14		0.33	0.40	0.27	0.33	0.38	0.42
0	3	3		3	0	3	3	5	9
1.00	0.71	0.50	0.33		0.09	0.16	0.23	0.28	0.33
0	4	0	3		1	7	1	6	3
1.00	0.75	0.55	0.40	0.09		0.07	0.14	0.20	0.25
0	0	6	0	1		7	3	0	0
0.75	0.55	0.40	0.27	0.16	0.07		0.06	0.12	0.17
0	6	0	3	7	7		7	5	6
0.77	0.60	0.45	0.33	0.23	0.14	0.06		0.05	0.11
8	0	5	3	1	3	7		9	1
0.80	0.63	0.50	0.38	0.28	0.20	0.12	0.05		0.05
0	6	0	5	6	0	5	9		3
0.81	0.66	0.53	0.42	0.33	0.25	0.17	0.11	0.05	
8	7	8	9	3	0	6	1	3	

JACCARDS

	0.50	0.66	0.75	1.00	1.00	0.85	0.87	0.88	0.90
	0	7	0	0	0	7	5	9	0
0.50		0.33	0.50	0.83	0.85	0.71	0.75	0.77	0.80
0		3	0	3	7	4	0	8	0
0.66	0.33		0.25	0.66	0.71	0.57	0.62	0.66	0.70
7	3		0	7	4	1	5	7	0
0.75	0.50	0.25		0.50	0.57	0.42	0.50	0.55	0.60
0	0	0		0	1	9	0	6	0
1.00	0.83	0.66	0.50		0.16	0.28	0.37	0.44	0.50
0	3	7	0		7	6	5	4	0
1.00	0.85	0.71	0.57	0.16		0.14	0.25	0.33	0.40
0	7	4	1	7		3	0	3	0
0.85	0.71	0.57	0.42	0.28	0.14		0.12	0.22	0.30
7	4	1	9	6	3		5	2	0
0.87	0.75	0.62	0.50	0.37	0.25	0.12		0.11	0.20
5	0	5	0	5	0	5		1	0
0.88	0.77	0.66	0.55	0.44	0.33	0.22	0.11		0.10
9	8	7	6	4	3	2	1		0
0.90	0.80	0.70	0.60	0.50	0.40	0.30	0.20	0.10	
0	0	0	0	0	0	0	0	0	

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	0.18	0.33	0.46	0.75	0.82	0.75	0.82	0.88	0.94
	2	3	2	0	4	0	4	9	7
0.18		0.18	0.33	0.66	0.75	0.66	0.75	0.82	0.88
2		2	3	7	0	7	0	4	9
0.33	0.18		0.18	0.57	0.66	0.57	0.66	0.75	0.82
3	2		2	1	7	1	7	0	4
0.46	0.33	0.18		0.46	0.57	0.46	0.57	0.66	0.75
2	3	2		2	1	2	1	7	0
0.75	0.66	0.57	0.46		0.18	0.33	0.46	0.57	0.66
0	7	1	2		2	3	2	1	7
0.82	0.75	0.66	0.57	0.18		0.18	0.33	0.46	0.57
4	0	7	1	2		2	3	2	1
0.75	0.66	0.57	0.46	0.33	0.18		0.18	0.33	0.46
0	7	1	2	3	2		2	3	2
0.82	0.75	0.66	0.57	0.46	0.33	0.18		0.18	0.33
4	0	7	1	2	3	2		2	3
0.88	0.82	0.75	0.66	0.57	0.46	0.33	0.18		0.18
9	4	0	7	1	2	3	2		2
0.94	0.88	0.82	0.75	0.66	0.57	0.46	0.33	0.18	
7	9	4	0	7	1	2	3	2	

APPENDIX2

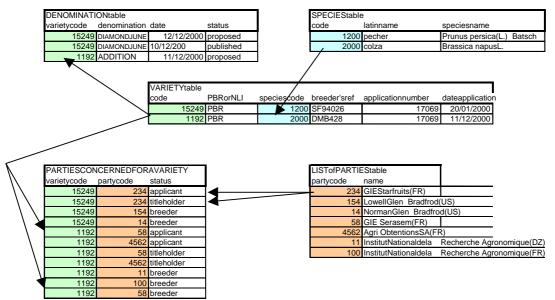
STORAGEOFINFORMATI ONINDIFFERENTWAYS :

EXTRACT FROM FILE SENT TO UPOV FOR CREATION OF THE UPOV - ROM (SGML FORMAT, INFORMATIONS ARE IDENTIFIED BYT AGS DESCRIBED INCIR CULARU 2462) ForinstanceTag190istoidentifyinformation"countrysendingtheinformation" <000>0 <190>FR <010>PBR 15249 <500>Prunus persica (L.) Batsch <510>Pecher <540>20001212 DIAMOND JUNE <541>20001210 DIAMOND JUNE <600>SF 94 026 <210>17069 <220>20000120 <400>20000210 <730>GIE Star Fruits (FR) <731>Lowell Glen Bradford (US) <731>Norman Glen Bradford (US) <733>GIE Star Fruits (FR) <000>0 <190>FR <010>PBR 1192 <500>Brassica napus L. <510>Colza <540>20001211 ADDITION <541> ADDITION <600>DMB 428 <210>17515 <220>20001211 <730>GIE Serasem (FR) <730>Agri Obtentions SA (FR) <731>Institut National de la Recherche Agronomique (DZ) <731>Institut National de la Recherche Agronomique (FR) <731>Serasem (FR) <733>GIE Serasem (FR) <733>Agri Obtentions SA (FR)

<u>Example of information kept in spreadsheet</u>: When there is more than one set of information kept for a variety, the maintenance of the file can become a problem, e.g. there are two co breeders invariety DIAMONDJUNE; 2co -applicants, 3co -breeders, 2co -titleholders for the BrassicanapusL.variety.

PBRor NLI	Identific ation species number name	Latinname			denominatio	denominati on accepted	breeder's reference	applicatio nnumber		Applicant's name	Breeder'sname	Titleholder's
PBR "	15249 Pecher	Prunus persica(L.) Batsch	12/12/2000	DIAMOND JUNE	02/10/2000	DIAMOND JUNE	SF94026	17069	20/01/2000	fruits(FR)		GIEStar fruits(FR)
PBR	1192 Colza	Brassica napusL. Brassica	11/12/2000	ADDITION		ADDITION	DMB428	17515	11/12/2000	GIE	InstitutNationaldela Recherche Agronomique(DZ) InstitutNationaldela	GIESerasem (FR) Agri obtentions
PBR PBR	1192 Colza 1192 Colza	napusL. Brassica napusL.		ADDITION		ADDITION ADDITION			11/12/2000	SA(FR)	Agronomique(FR) GIESerasem (FR)	SA(FR)

<u>Example of information kept in a database</u>: To avoid redundancy and to ease ma intenance, information is kept in a set of tables, for instance the name and address of a party is unique in the table LIST of PARTIES even if this party is used for dozens of varieties . Identification of appropriate party is done via a party code.



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