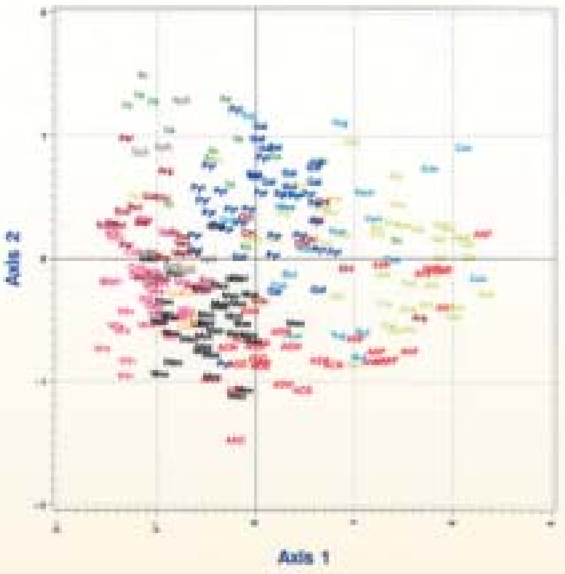
CIMMYT

User's Manual for the LCDMV Software (Calculation Software of Molecular Distances between Varieties)

For Fingerprinting and Genetic Diversity Studies



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Introduction:

LCDMV (in English, known as the Calculation Software of Molecular Distances between Varieties) is a computer program developed in the SAS language (SAS Institute Inc., version 6.12), with the help of the modules SAS-STAT, and SAS-IML. It was written to analyze biochemical markers (isozymes) or molecular markers (RFLP, STS, SSR, RAPD, AFLP) obtained on homogenous or heterogeneous varieties. Its main function is to estimate genetic distances between varieties, and to analyze the structure of the genetic makeup of a given collection of OTU's (Operational Taxonomic Units).

A. General Presentation and Organization of the Manual

The structure of the OTUs and the type of markers used are the main determining factors of the analysis method used by the program. We define two types of OTU structure: homogenous varieties, represented by clonal varieties, inbred lines and single cross hybrids, and heterogeneous varieties including double cross hybrids, three way crosses, and synthetic and traditional populations.

The markers were also classified into two distinct types: co-dominant markers, (isoenzymes, RFLPs, and SSRs) and dominant markers (RAPD and AFLP). In the first case, the marker bands can be defined to specific alleles, while in the second case, the allelic relationship between the markers is unknown. This program is designed to most efficiently use the information provided by each marker type in order to calculate distances most correctly. However, in order to standardize analyses as much as possible, dominant markers have been assumed in some cases to be co-dominant. In these cases, each marker (band) is assumed to be a dominant allele of a bi-allelic locus. Data entry for this program uses three structured files. Two of the files describe the OTUs (cultivars) and the markers, while the third file contains the marker data in a matrix of N lines and P columns; N being the individuals or populations (OTUs) analyzed, and P the number of genetic markers run on the collection of OTUs. Each line of the matrix consists of allelic frequencies of the markers of a given individual.

The program realizes a pre-determined series of calculations, depending on type of OTU and marker (dominant vs. co-dominant). In the case that markers are mixed (both dominant and co-dominant), all will be treated as dominant. The program can then be used to:

- Analyze the frequencies of alleles or bands in the collection of OTUs.
- Perform Principal Components Analysis (PCA). This will display the OTUs graphically either as populations or, if the populations were characterized with several individuals analyzed separately, they can be displayed individually on the graph.
- Choose the most appropriate estimator of genetic distance based on OTU/marker type combination.
- Estimate genetic distance between every pair of OTU in the study and the confidence interval for every estimate using either an analytic approach or an empirical approach using re-sampling (bootstrapping) if an analytical calculation is not possible.
- Display the histogram of genetic distances between all pairs of OTUs in the study, and a graph representing only pairs of OTUs for which the distance is lower than a threshold distance (as defined by the user)
- Perform a cluster analysis using the distance matrix and the method chosen by the user, and calculate the robustness of the dendrogram by bootstrapping if requested by the user.
- Create a chart of mapped loci that differ between two pure lines.

Generate a report file showing the polymorphic markers in the study, including information such as allele richness and diversity , as well as the estimated distances between every pair of OTUs and the confidence interval associated with those estimates.

B. Input file format

The LCDMV program uses 3 files saved in a text format:

a. The CULTIVARS file, giving a description of the OTUs.
b. The MARKERS file, giving the description of the markers.
c. The FRQUENCY file with the matrix of allele frequencies for each OTU.

B. 1 The CULTIVARS file

The CULTIVARS file describes the observations characterized in the FREQUENCES file, and contains 3 columns of variables:

- statut
- nom_var
- echant (without accent)

Note: These variables must be declared at the top of the file (on the first line) separated by at least one space or a tab. Spelling and case of the names must be consistent.

The STATUT variable is an alpha numeric variable allowing the precise specification of the cultivars (ie. reference or candidate). In the default version, the program does not use this variable, but it can be used in response to the future needs of the users who will choose to restrict the genetic distance calculations to the pairs (reference-reference) and (reference-candidate) for example, to limit the program's execution time, and to reduce the number of given results.

The NOM_VAR variable is an alpha-numeric variable used to identify the cultivars OTUs for analysis, either by commercial denomination, or by a code specified by the user. This variable is essential and must not be left blank. The name of an OTU may appear more than once in the file. In this case, the program treats each repetition as the same cultivar (or of the same data of a given cultivar) and the index automatically follows the order of this declaration. In example 1(below), the cultivar L3 is observed twice in the file (lines 4 and 7). Therefore, the first observation (line 4) will be given the suffix -1, while the second (line 7) will be given the suffix -2. The ECHANT variable is a numeric variable allowing the user to specify the number of individuals analyzed to characterize a heterogeneous cultivar. If the number of observations in the CULTIVARS file is equal to the number of observations in the FREQUENCES file, this variable is not required and left at the default, and the program automatically begins a chain of calculations suitable for homogenous varieties (pure lines or single cross hybrids). If on the other hand, the number of observations in the CULTIVARS file is less than to the number of observations in the FREQUENCES file, then the ECHANT variable is used to notify the program of the number of individuals to be analyzed for each of the cultivars (see example 2). This structure supposes that only one individual will suffice to correctly characterize the molecular profile of a homogenous variety, even if in practice, such homogenous varieties are generally characterized using several individuals separately or bulked.

Note: The data must be separated by at least one space or tab.

Example 1: A CULTIVARS file describing the observations of the FREQUENCES file.

statut	nom_var	echant ← Name of the variables in file head
r	**L1**	**1**
r	**L2**	**1**
r	**L3**	**1**
r	**L4**	**1**
r	**L5**	**1**
r	**L3**	**1**
r	**L7**	**1**

r	**L8**	**1**
r	**L9**	**1**
r	**L10**	**1**

Example 2: The CULTIVARS file describing the observations in the FREQUENCES file given in Example 2.

Statut	**nom_var**	**echant**
.	**Barlet**	**10**
.	**Barpolo**	**8**
.	**Barylou**	**6**

The variable "statut" must be specified. If you do not wish to specify this variable, use periods, as shown in the example here.

B. 2 The MARKERS file

The MARKERS file describes the markers corresponding to those in the FREQUENCES file, and up to, but no more than, 4 variables:

- locus
- allele
- chromo (specifying chromosome)
- pos_chr (specifying the position on the chromosome)

As in the case of the CULTIVARS file, these variables must be declared in the first line of the file, separated by at least one space or tab. They must remain consistent for spelling (including case sensitivity) and spaces. The LOCUS and ALLELE variables are necessary, and must not be left blank. The CHROMO and POS_CHR variables are optional, and should only be declared if the map data is available.

The LOCUS variable is alpha-numeric and identifies the locus of various markers (bands or alleles). In cases where the allele relationship between the markers is known, the identifier of a given locus should appear in the first column as many times as there are indexed alleles. In Example 1 below, the locus header ADH2-H is repeated 8 times to indicate each of its alleles, named 1 through 8. In cases where the allelic relationships between markers are unknown, each individual marker is identified by the locus variable, as if it were a separate locus (see Example 2 below).

The variable ALLELE identifies the alleles at each locus, by a number, a letter or by molecular weight (see Example 2). The variable CHROMO is a numeric variable used to identify the linkage group or chromosome of the analyzed locus. The variable POS_CHR is also numeric, and indicates the position of the locus on the linkage group or chromosome in centimorgans, compared to neighboring loci.
Note: The data must be separated by at least one space or tab.

Example 1: A MARKERS file describing the columns indicated in the FREQUENCES file given in Example 1 above, and assuming co-dominant loci with defined alleles.

Known map position

```
locus allele chromo pos_chr ←Name of the variables in file head
ADH2-H  1  4   9.7
ADH2-H  2  4   9.7
ADH2-H  3  4   9.7
ADH2-H  4  4   9.7
ADH2-H  5  4   9.7
ADH2-H  6  4   9.7
ADH2-H  7  4   9.7
ADH2-H  8  4   9.7
C10-H   B  3  54.3
C10-H   C  3  54.3
C10-H   A  3  54.3
C102-E  1  3  61.4
C102-E  2  3  61.4
C102-E  3  3  61.4
C102-E  4  3  61.4
C102-E  5  3  61.4
```

Unknown map position

locus allele ← Name of the variables in file head

ADH2-H	**1**
ADH2-H	**2**
ADH2-H	**3**
ADH2-H	**4**
ADH2-H	**5**
ADH2-H	**6**
ADH2-H	**7**
ADH2-H	**8**
C10-H	**B**
C10-H	**C**
C10-H	**A**
C102-E	**1**
C102-E	**2**
C102-E	**3**
C102-E	**4**
C102-E	**5**

Example 2: A MARKERS file describing the columns indicated in the FREQUENCES file given in Example 2, above, and assuming dominant loci (i.e., allelic relationships between bands do not exist or are not known).

locus	**allele**
Gen01	**72**
Gen02	**77**
Gen03	**83**
Gen04	**91**
Gen05	**10**
Gen06	**108**
Bar29	**277**
Bar30	**280**
Bar31	**284**
Bar32	**289**

AFLP Marker name

Molecular weight of the bands

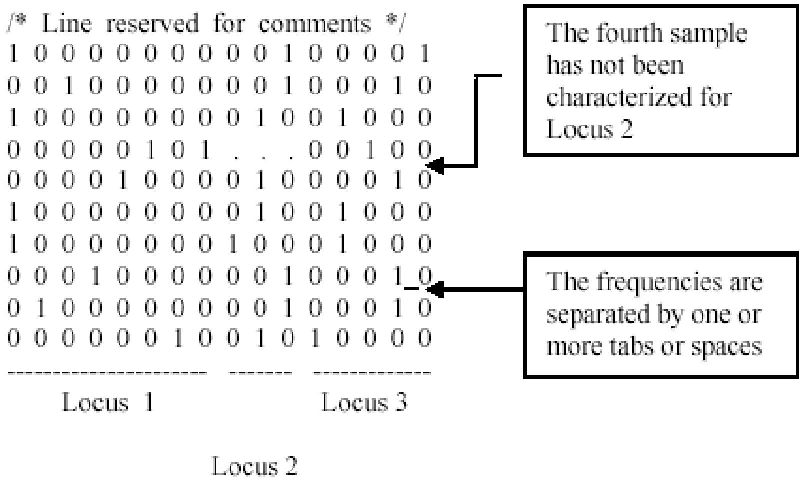
B. 3 The FREQUENCES file

The FREQUENCY file contains as many observations (lines) as there are individuals (OTUs) analyzed to characterize a population, as described in the CULTIVARS file. This value will be one, if it is a pure line cultivar or single cross hybrid. It also contains as many variables (columns) as the number of markers (described in the MARKERS file) characterizing these OTUs. Each line corresponds to the frequency profile of the markers of each given individual. Notice that these frequencies are discrete: 0, 0.5 or 1 in each diploid individual; 0, 0.25, 0.5, 0.75 or 1 in each tetraploid individual, etc. for higher ploidy levels.

Note: The data must be separated by a space or a tab, and missing data indicated by a period (.). The first record should start on the second line, and the first line is reserved for a description of the file (origin and type of data), written as a SAS comment, that is, beginning with the /* symbols

and ending with the */ symbols

Example 1: FREQUENCES file of 10 homogenous varieties (10 pure line OTUs) characterized by 3 loci having 8, 3 and 5 alleles each (with a total of 16 variables). Note that the frequencies of the alleles at each locus within an individual must sum to 1. Because these are pure lines, only homozygous states (0 or 1) are recorded in this example.



```
/* Line reserved for comments */
1 0 0 0 0 0 0 0 0 0 1 0 0 0 0 1
0 0 1 0 0 0 0 0 0 0 1 0 0 0 1 0
1 0 0 0 0 0 0 0 0 1 0 0 1 0 0 0
0 0 0 0 0 1 0 1 . . . 0 0 1 0 0
0 0 0 0 1 0 0 0 0 1 0 0 0 0 1 0
1 0 0 0 0 0 0 0 0 1 0 0 1 0 0 0
1 0 0 0 0 0 0 0 1 0 0 0 1 0 0 0
0 0 0 1 0 0 0 0 0 0 1 0 0 0 1 0
0 1 0 0 0 0 0 0 0 0 1 0 0 0 1 0
0 0 0 0 0 0 1 0 0 1 0 1 0 0 0 0
----------------------  -------  -------------
      Locus 1                      Locus 3

                  Locus 2
```

The fourth sample has not been characterized for Locus 2

The frequencies are separated by one or more tabs or spaces

Example 2: FREQUENCES file for 3 heterogeneous varieties characterized by 10 AFLP markers; numbers of individuals (lines) per population were defined in the CULTIVARS file showed in example 2.

```
--Line reserved for comments-
1	1	1	0	1	1	1	1	0	1
1	1	1	0	1	1	1	1	0	1
1	1	1	1	1	1	1	1	0	1
1	0	1	0	1	1	1	1	0	1
1	1	1	0	1	1	1	1	0	1
1	0	1	0	1	1	1	1	0	1
0	1	1	0	1	1	1	1	1	1
1	1	1	1	0	1	1	1	0	1
1	1	1	1	1	1	1	1	0	1
1	1	1	1	1	1	1	1	1	1
1	1	1	0	1	1	1	1	0	1
1	1	1	1	1	1	1	1	0	1
1	1	1	0	1	0	1	1	0	1
0	1	1	0	1	1	1	1	0	1
1	1	1	0	1	1	1	1	0	0
1	1	1	0	1	1	1	1	0	1
0	1	1	0	1	1	1	1	0	1
1	1	1	0	1	1	1	1	1	0
1	1	0	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1
1	1	1	0	1	1	0	1	1	1
1	1	0	0	1	1	1	1	0	1
1	1	1	0	1	1	1	1	0	1
1	1	1	0	1	1	1	1	1	1
```

Variety 1 (10 indivuals)

Variety 2 (8 individuals)

Variety 3 (6 individuals)

The data is separated by tabs.

C. Analyses performed by the program

C.1 Genetic diversity measurements

Regardless of the type of OTU, the program estimates:

1. in the case that the molecular information can be classified as <<locus-allele>>
   - The number of alleles per locus in this study
   - Nei's diversity index for populations in panmixis over all loci in the study.

$H_e^l = 1 - \sum_{a=1}^{A_l} \left(\hat{P}_{al}\right)^2$ (Nei, 1973), Where A_l equals the total number of alleles for locus *l*, and $\hat{P}_{al}$, the estimated frequency of allele *a* at locus *l* in the current study.

2. in the case that molecular information can be classified as « bands »,
   - The frequency of the markers (bands) in the study.
   - The PIC (Polymorphism Information Content) of the markers, which is equivalent to Nei diversity estimate for biallelic loci:

$$PIC = 2\hat{p}_m(1-\hat{p}_m),$$

Where $\hat{P}_m$ is the estimated frequency of the marker *m* in the study.

If the collection is composed of heterogeneous varieties, the program estimates:

- The average number of individuals characterized by locus and by variety.
- The mean number of alleles within each variety over all loci in the study.
- The Nei diversity index (markers « locus-alleles ») or PIC value (markers « bands ») within each variety in the study:

$$H_e^i = \frac{1}{L}\sum_{l=1}^{L}\left[\frac{2n_l}{2n_l-1}\left(1-\sum_{a=1}^{A_l}\left(\hat{P}_{al}^i\right)^2\right)\right] \text{ (Nei, 1978),}$$

Where *L* is the total number of loci characterized for variety *i*, n_l is the number of individuals characterized for locus *l,* and $\hat{P}_{al}$ is the estimated frequency of allele *a* at locus *l* for variety *i.*

C.2 Principal Components Analyses (PCA)

1. Homogenous Varieties:

The program will perform a PCA on the matrix of genetic distances calculated from the frequencies of the markers (alleles or bands) stored in the FREQUENCES file. Missing data is replaced by the frequency of the markers within all varieties in the study .

The quality of the graphical representation of an OTU is estimated by the square of the cosine of the angle θ between the original vector in the space represented by the centered and reduced variables (bands or alleles) and its projection on the principal axis (under 2 spatial dimensions). This approaches one as the area between the 2 vectors becomes very small ($Cos^2\theta \to 1$ when $\theta \to 0$). It is graphically symbolized by a circle (centered on the variety).

2. Heterogenous Varieties:

The program performs two different Principal Components Analyses for heterogeneous varieties:

- A PCA on the correlation matrix of the frequencies of the markers (alleles or bands) estimated within each population. The graphical output is calculated and represented as in C.2.1, homogenous varieties.

- A PCA on the correlation matrix of the frequencies of the markers with the individuals of each population analyzed separately (individual data of the FREQUENCES file). The missing data are estimated as in C.2.1. The Euclidian center of the cloud of points formed by the individuals in each population is calculated, and represented in a graphic with together all of the individual points.

C.3 Estimation of the genetic distances and precision of the estimates

1. Homogenous variety – «locus-allele» markers:

Squared Modified Rogers (1972) distance estimator is used by the program:

$$D_R^{ij} = \frac{1}{2L}\sum_{l=1}^{L}\sum_{a=1}^{A_l}\left(P_{al}^{i} - P_{al}^{j}\right)^2 ,$$

Where *L* is the total number of loci characterizing the *i* and *j* varieties being compared; A_l,, is the number of observed alleles at locus *l* in the collection; and P_{al}^{i} (P_{al}^{j}), the frequency of allele *a* of locus *l* in the variety *I (j)*. In the case where *i* and *j* are pure lines, the Squared Modified Rogers distance estimates the percentage of the loci for which the lines differ.

The sampling variance of the Squared Modified Rogers distance is estimated by:

$$Var(D_R^{ij}) = \frac{D_R^{ij}(1 - D_R^{ij})}{L} \qquad \text{(Dillmann, 1997).}$$

- If the collection of OTUs is made up of simple hybrids, or the varieties are incompletely fixed lines (ie, residual heterozygosity for at least one locus in at least one line) the approximate boundaries of the Squared Modified Rogers distance confidence intervals are estimated by assuming a normal distribution of the distance:

$$Inf(D_R^{ij}) = D_R^{ij} - u_\alpha \sqrt{Var(D_R^{ij})}\ , \quad \text{and}$$

$$Sup(D_R^{ij}) = D_R^{ij} + u_\alpha \sqrt{Var(D_R^{ij})}\ ,$$

with u_α, the Z-value by the normal standard table for a chosen probability level α.

- If the collection of OTUs to be analyzed is solely made up of pure line varieties (ie, homozygous for all the loci in all the lines), and if we assume that the loci are independent and unlinked, the Rogers distribution follows the binomal distribution $B(L, D_R^{ij})$. In this case, the exact boundaries of the Squared Modified Rogers distance confidence interval is calculated according to Collett, (1991) :

$$Inf(D_R^{ij}) = \frac{LD_R^{ij}}{LD_R^{ij} + \left[L\left(1 - D_R^{ij}\right) + 1\right]F_{(1-\alpha/2)\left[2\left[L(1-D_R^{ij})+1\right];2LD_R^{ij}\right]}}, \quad \text{and}$$

$$Sup(D_R^{ij}) = \frac{LD_R^{ij} + 1}{\left(LD_R^{ij} + 1\right) + L\left(1 - D_R^{ij}\right)F_{(\alpha/2)\left[2L(1-D_R^{ij});2(LD_R^{ij}+1)\right]}}$$

- If the varieties are pure lines, and the CHROMO and POS_CHR variables of the MARKERS file have been given for at least a part of the locus (Cf. B.3), the program estimates in addition to the Squared Modified Rogers distance, the BLUE (Best Linear Unbiaised Estimator) of the Squared Modified Rogers distance (Dillmann *et al.* 1997) estimated from the mapped loci:

$$\tilde{D}_B^{ij} = \left(1'V^{-1}1\right)^{-1}(1'V^{-1})D_R^{ij}$$

Where *1* is the dimension identity of the vector (L^*,1) ; D_R^{ij} (={0,1}),the Squared Modified Rogers distance vector estimated for L^* individual locus between the *i* and *j* varieties; and *V* is the variance-covariance of the Squared Modified Rogers distance for the individual locus, estimated by the map distance between locus.

The $\widetilde{D}_B^{ij}$ variance is estimated by:

$$Var(\widetilde{D}_B^{ij}) = \left(1'V^{-1}1\right)^{-1}.$$

The approximate boundaries of the confidence intervals of the Squared Modified Rogers distances observed are estimated under the normality hypothesis of $\widetilde{D}_B^{ij}$:

$$Inf(\widetilde{D}_B^{ij}) = \widetilde{D}_B^{ij} - u_\alpha \sqrt{Var(\widetilde{D}_B^{ij})} \text{ and,}$$

$$Sup(\widetilde{D}_B^{ij}) = \widetilde{D}_B^{ij} + u_\alpha \sqrt{Var(\widetilde{D}_B^{ij})}$$

2. Homogenous variety – «band» markers:

This distance estimator used in this case is that of Nei and Li (1979):

$$D_N^{ij} = 1 - \frac{2N^{ij}}{N^i + N^j},$$

Where N^{ij} is the number of common bands between varieties i and j; and N^i (N^j), the number of bands for the variety i (j). For information only, the distance of Jaccard (1900; 1908) is deducted from the distance of Nei&Li:

$$D_J^{ij} = \frac{2D_N^{ij}}{1 + D_N^{ij}} \text{ (Snijders } et\ al.\text{, 1990)}$$

The variance of the Nei and Li distance is estimated by:

$$Var(D_N^{ij}) \approx 4\left[\frac{N^{ij}\left(1 - 2N^{ij}/m\right)}{m^2} + \frac{v(N^{ij})^2}{m^4}\right] \text{, (Dillmann, 1999; pers. com.)}$$

Where m is the average and v is the variance of the total number of bands present within every pair of varieties within the study.

$$m = \frac{2}{n_V(n_V - 1)} \sum_{i=1}^{n_V} \sum_{j>i}^{n_V} \left(N^i + N^j\right) \text{, and}$$

$$v = \frac{2}{n_V(n_V - 1)} \sum_{i=1}^{n_V} \sum_{j>i}^{n_V} \left(N^i + N^j\right)^2 - m^2$$

Where n_V equals the number of varieties in the study.

This estimate assumes that N^{ij} follows a binomial distribution with N and P calculated as:

$$N = \frac{E(N^i + N^j)}{2} \approx \frac{m}{2}, \text{ and } \quad P = \frac{N^{ij}}{N} \approx \frac{2N^{ij}}{m}$$

The approximate confidence interval of the Nei and Li distance is estimated as:

$$Inf(D_N^{ij}) = D_N^{ij} - u_\alpha \sqrt{Var(D_N^{ij})}, \text{ and } Sup(D_N^{ij}) = D_N^{ij} + u_\alpha \sqrt{Var(D_N^{ij})}.$$

If requested by the user, the program will estimate the confidence interval using the values observed following repeated sampling with replacement (the bootstrap procedure). This method is recommended, as the analytical estimation can become very imprecise for small sample sizes.

3. Heterogenous varieties – «locus-allele» markers:

Two types of distances can be used by the program: Squared Modified Rogers distance (1972) and Sanghvi (Foulley and Hill, 1999).

The Squared Modified Rogers distance estimate is calculated by:

$$\widetilde{D}_R^{ij} = \frac{1}{2L}\sum_{l=1}^{L}\sum_{a=1}^{A_l}\left(\hat{P}_{al}^i - \hat{P}_{al}^j\right)^2 - \frac{1}{2L}\sum_{l=1}^{L}\sum_{\substack{a=1\\a'>a}}^{A_l}\left(\frac{\hat{P}_{al}^i\hat{P}_{a'l}^i}{N^i - 1} + \frac{\hat{P}_{al}^j\hat{P}_{a'l}^j}{N^j - 1}\right),$$

Where L is equal to the number of loci characterizing the varieties i and j, $\hat{P}_{al}^i$ ($\hat{P}_{al}^j$), is the estimated frequency of allele a at the locus l in the variety i (j), and N^i (N^j) is the number of individuals in the variety i (j).

The confidence interval is estimated using the values observed following repeated sampling with replacement (the bootstrap procedure). To remove bias from the estimation of the distance which can occur in this resampling of individuals within varieties, the distance is recalculated as:

$$\widetilde{D}_R^{i^*j^*} = \frac{1}{2L}\sum_{l=1}^{L}\sum_{a=1}^{A_l}\left(\hat{P}_{al}^{i^*} - \hat{P}_{al}^{j^*}\right)^2 - \frac{1}{2L}\sum_{l=1}^{L}\sum_{\substack{a=1\\a'>a}}^{A_l}\left(\frac{\hat{P}_{al}^i\hat{P}_{a'l}^i}{N^i - 1} + \frac{\hat{P}_{al}^j\hat{P}_{a'l}^j}{N^j - 1} + \frac{\hat{P}_{al}^{i^*}\hat{P}_{a'l}^{i^*}}{N^i - 1} + \frac{\hat{P}_{al}^{j^*}\hat{P}_{a'l}^{j^*}}{N^j - 1}\right),$$

Where $\hat{P}_{al}^{i^*}$ ($\hat{P}_{al}^{j^*}$) is the estimated frequency allele a has at the locus l in the bootstrap values of i^* (j^*) for the variety i (j).

The Sanghvi distance estimate is calculated by:

$$\hat{D}_S^{ij} = \frac{1}{A_+ - L}\sum_{l=1}^{L}\sum_{a=1}^{A_l}\frac{\left(\hat{P}_{al}^{i} - \hat{P}_{al}^{j}\right)^2}{\hat{P}_{al}},$$

Where $A+$ is equal to the total number of alleles on the sample of the L loci analyzed, and $\hat{P}_{al}$, the estimated frequency of allele a at the locus l in the variety sampled.

The variance of the Sanghvi distance estimate is calculated according to Foulley and Hill (1999):

$$Var(D_S^{ij}) = \frac{1}{\left(A_+ - L\right)^2}\sum_{l=1}^{L} 2\left(A_l - 1\right)\left(\hat{D}_S^{ijl} + 1/N\right)^2,$$

Where $\hat{D}_S^{ijl}$ is the estimated Sanghvi distance for locus l between the varieties i and j, and N is the harmonic average of the number of individuals in the varieties i and j. N is calculated by :

$$N = \frac{1}{2\left(\frac{1}{N^i} + \frac{1}{N^j}\right)}.$$

4. Heterogenous varieties – «band» markers:

These distances are calculated using Rogers (1972) calculation, which estimates distance as:

$$\tilde{D}_R^{ij} = \frac{1}{M}\sum_{m=1}^{M}\left(\hat{P}_m^i - \hat{P}_m^j\right)^2 - \frac{1}{M}\sum_{m=1}^{M}\left[\frac{\hat{P}_m^i\left(1-\hat{P}_m^i\right)}{N^i - 1} + \frac{\hat{P}_m^j\left(1-\hat{P}_m^j\right)}{N^j - 1}\right] \text{ (Ghérardi and al., 1998),}$$

Where M is equal to the total number of markers (bands), $\hat{P}_m^i$ ($\hat{P}_m^j$) is the estimated frequency of the marker m within the variety i (j), and N^i (N^j) is the number of individuals in the variety i (j).

Note: this distance is estimated under the hypothesis that every marker (band) is a dominant allele of a biallelic locus. This hypothesis is acceptable in the case of dominant marker types, such as RAPD or AFLP. Use of this distance estimator will be incorrect in the cases of codominant markers scored merely as present or absent, because knowledge of the genetic relationships among alleles will be missing from the program. In these cases, the Squared Modified Rogers distance calculated here will overestimate the distance between varieties.

The confidence interval is estimated using the values observed following repeated sampling with replacement (the bootstrap procedure). To remove bias from the estimation of the distance that can occur in this resampling of individuals within varieties, the distance is recalculated as:

$$\widetilde{D}_R^{i^*j^*} = \frac{1}{M}\sum_{m=1}^{M}\left(\hat{P}_m^{i^*} - \hat{P}_m^{j^*}\right)^2 - \frac{1}{M}\sum_{m=1}^{M}\left(\frac{\hat{P}_m^{i}\left(1-\hat{P}_m^{i}\right)}{N^i - 1} + \frac{\hat{P}_m^{j}\left(1-\hat{P}_m^{j}\right)}{N^j - 1} + \frac{\hat{P}_m^{i^*}\left(1-\hat{P}_m^{i^*}\right)}{N^i - 1} + \frac{\hat{P}_m^{j^*}\left(1-\hat{P}_m^{j^*}\right)}{N^j - 1}\right),$$

Where M is equal to the total number of markers, $\hat{P}_m^i$ ($\hat{P}_m^j$) is the estimated frequency of the marker *m* in the initial sample of the variety *i(j),* and $\hat{P}_m^{i^*}$ ($\hat{P}_m^{j^*}$), the frequency of the marker *m* in the resampling of the variety *i (j).*

C.4. Hierarchical Classification

To visualize the results of the distances in the matrices, the program will perform a hierarchical classification, using four different methods for the user to choose from. Details of the methods can be found in Bouroche and Saporta, (1980). Formulas to calculate the distances between groups formed in the preceding steps and any given element *k* using the following four methods:

- UPGMA (Unweighted Pair Group Average Method)

  $d[k,(i \cup j)] = p_i d(i,k) + p_j d(j,k)$,

  where $p_i = n_i /(n_i + n_j)$ (weights assigned to group *i*) and,

  $p_j = n_j /(n_i + n_j)$ (weights assigned to group *j*).

- Ward (minimum variance within groups)

  $d[k,(i \cup j)] = (p_k + p_i)d(k,i) + (p_k + p_j)d(k,j) - p_k d(i,j)$,

  where $p_k = n_k /(n_k + n_i + n_j)$ (weights assigned to group *k*),

  $p_i = n_i /(n_k + n_i + n_j)$ (weights assigned to group *i*), and

  $p_j = n_j /(n_k + n_i + n_j)$ (weights assigned to group *j*).

- Minimum (nearest neighbor)

  $d[k,(i \cup j)] = \min[d(k,i), d(k,j)]$

- Maximum (furthest neighbor)

  $d[k,(i \cup j)] = \max[d(k,i), d(k,j)]$

If the user requests it, a bootstrap procedure will be used by the program to test the stability of the junctions in the original dendrogram. The stability of these junctions or join points is estimated by the percentage of times where the varieties joined by this junction in the original dendrogram are grouped together in the dendrograms calculated by each resample during the bootstrap procedure:

$$R_d^c = \frac{(100N_{d^*}^c)}{N_{d^*}},$$

Where R_d^c is the stability of the junction *c* in the original dendrogram *d*, $N_{d^*}^c$ is the number of times where junction *c* is preserved among the trees produced in each resample, and N_{d^*} is the total number of resampling done in the bootstrap.

Note: the calculations of stability of the junctions are considered only for dendrograms constructed using Rogers and Nei and Li distances.

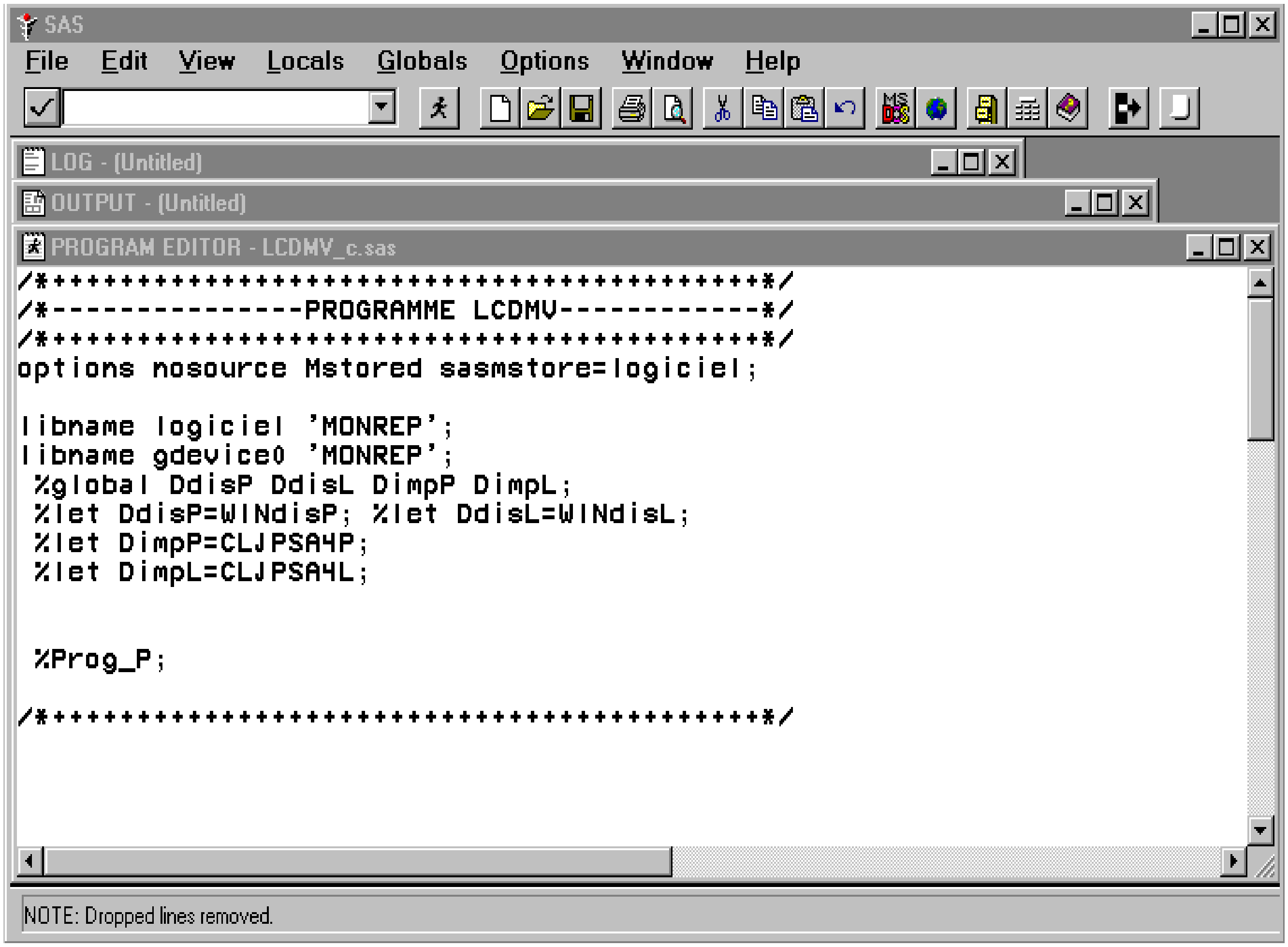
D. Installation of LCDMV and practice session

To use LCDMV, you must have a recent version of SAS (later than version 6.10) running on a PC or Unix, installed with the IML (Interactive Matrix Language). The complete LCDMV software package contains:

1. A catalog of macros called Sasmacro.Sc2
2. A catalog of devices called Devices.Sc2
3. A permanent SAS file called Varstore.Sd2
4. A SAS program called Lcdmv.sas

These four elements are required, and must be stored in the same folder (for example, c:\logiciel\Lcdmv). When these elements have been copied to the folder of your choice, you must open the program Lcdmv.sas (menu Spins-orders Open) in interactive method (the windows PROGRAM EDITOR, OUTPUT, and LOG must be accessible) as below, and replace MONREP by the name of the path and folder under which you have saved the four elements of the LCDMV program (formerly : C: \logiciel\Lcdmv).

Note: Modifying any other part of this file could result in errors or loss of function.



```
/*+++++++++++++++++++++++++++++++++++++++++++*/
/*---------------PROGRAMME LCDMV------------*/
/*+++++++++++++++++++++++++++++++++++++++++++*/
options nosource Mstored sasmstore=logiciel;

libname logiciel 'MONREP';
libname gdevice0 'MONREP';
 %global DdisP DdisL DimpP DimpL;
 %let DdisP=WINdisP; %let DdisL=WINdisL;
 %let DimpP=CLJPSA4P;
 %let DimpL=CLJPSA4L;


 %Prog_P;

/*+++++++++++++++++++++++++++++++++++++++++++*/
```

NOTE: Dropped lines removed.

In order to use the C:\logiciel\lcdmv path as the default path you must do the next changes:

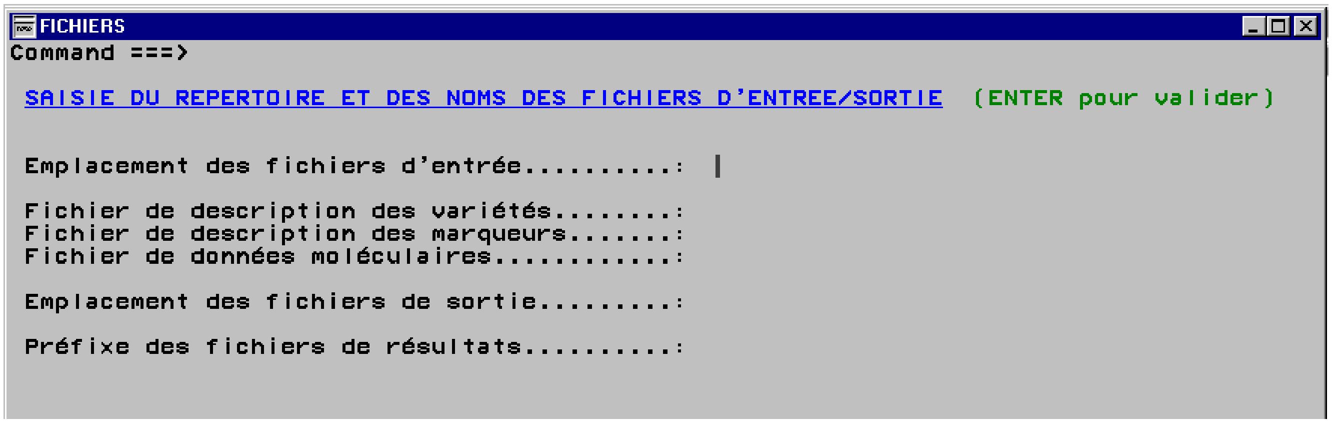
```
/*---------------------PROGRAMME LCDMV---------------------*/
/*++++++++++++++++++++++++++++++++++++++++++++++*/
options nosource Mstored sasmstore=logiciel;


Libname logiciel 'C:\logiciel\lcdmv';';
Libname logiciel 'C:\logiciel\lcdmv';
 %global DdisP DdisL DimpP DimpL;
 %let DdisP=WINdisP; %let DdisL=WINdisL;
 %let DimpP=CLJPSA4P;
 %let DimpL=CLJPSA4L;
```

%Prog_P;

/*++*/

Save the modification that you have made (menu Spins – orders Knows; or by clicking the save button) and run the program with the Submit command from the Local menu or by clicking the following icon:

When the program has finished running successfully, you should see the FILES window appear within a few seconds:

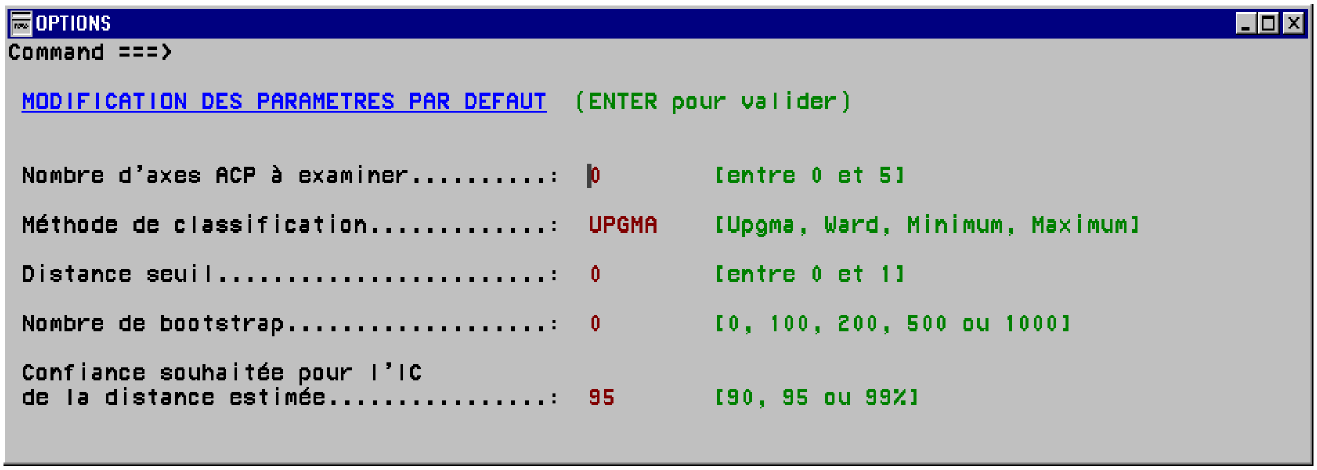


```
Command ===>

 SAISIE DU REPERTOIRE ET DES NOMS DES FICHIERS D'ENTREE/SORTIE  (ENTER pour valider)

 Emplacement des fichiers d'entrée..........:

 Fichier de description des variétés........:
 Fichier de description des marqueurs.......:
 Fichier de données moléculaires............:

 Emplacement des fichiers de sortie.........:

 Préfixe des fichiers de résultats..........:
```

Indicate the following:

1. The complete path under which you saved the three files necessary to run the software (for example: C: \logiciel\lcdmv\files\maïs\RAPD),
2. the name of the 3 entry files (for example, V_rapd. dat, M_rapd. dat, and P_rapd.dat),
3. The complete path under which you wish the resulting files to be stored (for example: C:\logiciel\lcdmv\results\maïs\RAPD), and
4. the prefix that you wish to give the results file, to distinguish them from the input file (for example, RAPD).

Upon successful completion of this step, you will see the following OPTIONS window appear:



```
OPTIONS
Command ===>

 MODIFICATION DES PARAMETRES PAR DEFAUT  (ENTER pour valider)

 Nombre d'axes ACP à examiner..........:  0        [entre 0 et 5]

 Méthode de classification.............:  UPGMA    [Upgma, Ward, Minimum, Maximum]

 Distance seuil........................:  0        [entre 0 et 1]

 Nombre de bootstrap...................:  0        [0, 100, 200, 500 ou 1000]

 Confiance souhaitée pour l'IC
 de la distance estimée................:  95       [90, 95 ou 99%]
```

You then can validate the data using the default options or continue to modify the data by the following:

- Perform a PCA, while specifying the number of axes that you wish to examine (up to five).
- Change the classification method to something other that UPGMA (Ward, for example).
- Display the varieties on a different scale, for example to show them grouped more closely together by specifying a larger distance threshold.
- Calculate a confidence interval on the genetic distance estimates, or test the robustness or stability of the join points of the dendrogram by performing a bootstrap analysis and specifying the number of resampling to use.
- Choose the confidence level of the confidence interval of the estimated genetic distances.

Upon choosing one of these analysis options, the program begins calculations and progressive editing of the output. The output graphic will depend on the analysis chosen, and the combination of variety type and marker type, as identified by the program and specified by your input files. During the execution of the program, you may wish to move to the GRAPH window to see the output. If you wish to examine in detail or to modify an output graph (for example, to change the legend), you can use the command Edit Graph from the Edit menu or by clicking on following icon:

The End command of the Spins menu (graphic editor) allows you to return to the GRAPH window. You then can decide to print the graph or to save it to a file. To do so, go to the Spins menu and chose the Print command. Export the file to be printed; by default, the graphs are in a postscript (.ps) format. Only printed postscript copies can guarantee you the same format; using other options may give random results and you will need to check the settings on your printer. However, by Exporting the file, you will have different options for saving the graphics.

To verify the results of the analyses performed by the program, check the file created by the program (it will be named “prefix.out” where prefix is the name of your input file). You will find 3 to 4 text files corresponding to the files specifying variety type of the cultivars analyzed and the type of marker used. The prefix you use in naming the files is followed by a number in order of their creation; ie, RAPD_1, RAPD_2, …).

Without exiting the SAS program, you can recall the program Lcdmv.sas (Local menu – Recall text command) which will place you in the PROGRAM EDITOR window. This will relaunch the execution of the program. Because files are edited progressively as you work and saved sequentially in the folder WORK.GSEG that already contains the graphs created in the first session, you will soon have many files in this folder. However, these files are automatically destroyed the moment you exit the SAS program. You must remember to print or save the graphs you wish to keep using the Print or Export commands from the Spins menu.

F. References

Bouroche JM et G Saporta (1980) L'analyse des données. Que sais-je. PUF.

Collet D (1991) Modeling binary data. London : Chapman & Hall, pp. 23-25.

Dillmann C., A. Charcosset, B. Goffinet, JSC Smith et Y. Dattée (1997) Best linear unbiased estimator of the molecular genetic distance between inbred lines. Advances in Biometrical Genetics. Proceedings of the Tenth Meeting of EUCARPIA Section Biometrics in plant Breeding, Posnań, 14-16 May 1997, P. Krajewski and Z. Kaczmarek (eds.), pp. 105-110.

Foulley JL et WG Hill (1999)A propos de l'estimation de la précision d'estimation de la distance génétique. XXXI Journées de Statistiques. 17-21 Mai 1999, Grenoble. Session Biométrie et Génome.

Ghérardi M, B Mangin, B Goffinet, D Bonnet, T Huguet (1998) A method to measure genetic distance between allogamous populations of alfalfa (*Medicago sativa*) using RAPD markers. Theor. Appl. Genet. 96 :406-412.

Jaccard P (1900) Contribution au problème de l'immigration post-glaciaire de la flore alpine. Bulletin de la Société Vaudoise des Sciences Naturelles 37 :547-579.

Jaccard P (1908) Nouvelles recherches sur la distribution florale. Bulletin de la Société Vaudoise des Sciences Naturelles 44 :223-270.

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70 :3321-3323.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89 :583-590.

Nei M et WH Li (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76 :3269-3273.

Rogers JS (1972) Measures of similarities and genetic distances. Studies in genetics VII. Univ Texas Publ 7213 :145-153.

Snijders TAB, M Dormaar, WH van Schuur, C Dijkman-Caes et G Driessen (1990) Distribution of some similarity coefficients for dyadic binary data in the case of associated attributes. J Clas 7 :5-31.

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