Evaluation of genetic variability among a French reference collection of inbred sunflower lines by RFLPs

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Abstract

In a context of development of molecular markers for helping the DUS (Distinctness, Uniformity and Stability) testing, RFLP tool was explored to assess the genetic variability in a French reference collection of sunflower inbred lines. Forty-six inbred lines of sunflower representing the whole morphophysiological variability observed within the reference collection of GEVES were selected for this study. The genetic variation among these 46 lines was assessed with 42 pre-screened cDNA clones, combined with *Hin*dIII or *Eco*RI. On average, 4.8 fragments and 5.8 profiles were produced per probe-enzyme combination. Both similarity index *F* and distance *d* (Nei and Li, 1979) were estimated from RFLP data to study the relationships among the sunflower inbred lines. The *F* values ranged from 0.43 to 0.98. The UPGMA dendrogram obtained as well as the results of principal component analysis showed a good separation of the maintainer lines from the restorer lines. The grouping of the lines from the two clustering analyses had a good concordance with the observations in the field. The average gene diversity computed with 42 probe-enzyme combinations was 0.63 (S.E. = 0.083). The results obtained suggests that RFLPs could be very useful descriptors for sunflower inbred line and variety studies when sufficient number of polymorphic probes can be used.

Key-Words: sunflower, inbred lines, French reference collection, genetic variability, RFLP (Restriction Fragment Length Polymorphism)

Introduction

Assessment of the distinctness of new varieties of plants is the basis for granting the protection of plant breeder's right. At present, the variety description and identification is essentially based on the investigation of morphophysiological characters according to UPOV (Union internationale pour la Protection des Obtentions Végétales) convention. This work is becoming more and more difficult in certain important crops such as sunflower (*Helianthus annuus)* due to the drastic increasement of the number of applications under test and their narrow genetic basis, and the great susceptibility of the

expression of morphological characteristics to enviromental conditions. For this reason, isozymes have been developed to help the description and identification of inbred lines as well as for checking the pedigree of hybrids in sunflower (Quillet et al. 1992, Bourgoin-Grenèche and Lallemand, 1993). However, the poor number of polymorphic enzyme loci available in sunflower and the low level of polymorphism per locus limit their utility in line description. More recently, RFLPs have showed a very good discriminant power as genetic descriptor in sunflower (Berry, et al., 1994, Gentzbittel et al., 1994, Zhang et al., 1995). The objective of the present study was (1) to assess the genetic variability in a French reference collection of sunflower inbred lines by RFLPs, and (2) to evaluate the potentials of RFLPs as genetic markers for the description and identification of sunflower inbred lines.

Materials and methods

Forty-six inbred lines of sunflower, bred by different public and private organisms (public and protected lines), were selected for this study. They represent the whole morphophysiological variability observed among the sunflower inbred lines maintained in the French reference collection in GEVES (Groupe d'Etude et de Contrôle de Variétés et de Semences) which is an official institute responsible for DUS testing. Among these lines, several pairs are very close or not differentiated by morphological characters. For reason of confidentiality, the lines used for this study were coded from number 1 to 46; lines 1 to 25 are maintainer (M) lines and lines 26 to 46 are restorer (R) lines.

The techniques used for DNA isolation as well as for RFLP analyses have been previously described (Gentzbittel et al., 1994). RFLP profiles in autoradiographs were scored visually. The presence or absence of a band in a gel line was coded by 1 or 0 respectively.

Both similarity index F and distance *d*, expressed as mean number of nucleotide substitutions per nucleotide site (Nei and Li, 1979) were estimated from RFLP data to study the relationships among the sunflower inbred lines. Likewise, the dendrogram of the sunflower inbred lines was generated from the distance index *d* according to UPGMA method. For grouping the sunflower inbred lines, the principal component analysis (PCA) was also performed using the Nei'*F* matrix.

Results

Genetic variation among the 46 inbred lines of sunflower was assessed with 42 cDNA clones, combined with *Hin*dIII or *Eco*RI. All the 42 clones were pre-screened and detected polymorphism on an another set of sunflower inbred lines (Gentzbittel et al., 1994). The criteria of the choice of these clones were (1) genome coverage (Gentzbittel et al., 1995), (2) hybridisation quality and (3) polymorphic content.

A total of 203 fragments were detected by the 42 probe-enzyme combinations, corresponding an average of 4.8 fragments per probe-enzyme combination. The number of fragments detected per probe-enzyme combination varied from 2 to 9. The 42 probe-enzyme combinations produced 246 RFLP profiles across the 46 inbred lines of sunflower,

with an average of 5.8 profiles per probe-enzyme combination. The number of RFLP profiles per probe-enzyme combination ranged from 2 to 17.

The average gene diversity (H) (Nei, 1987) was estimated with the 42 probe-enzyme combinations. It was 0.63 (S.E. = 0.083).

Nei and Li's *F* index as well as Nei's distance *d* were calculated for all the 1035 possible pairwise comparisons between the 46 sunflower inbred lines. The *F* values ranged from 0.43 (for lines 4 and 31) to 0.98 (for lines 1 and 2). Out of the 1025 possible pairwise combinations, 19 pairs of lines had a *F* value more than 0.80 ; 7 pairs of lines had a *F* value more than 0.90 (Table 1). The estimates of the distance *d*, expressed in mean number of nucleotide substitutions per nucleotide site, varied from 0.01 to 0.50.

Based on the RFLP distances estimated, an UPGMA dendrogram showing the relationships between the 46 inbred lines of sunflower was constructed (Fig. 1). Two big branches can be observed on the dendrogram: on the top a branch of the R lines and on the bottom a branch of M lines and there are several subgroups among each principal group. However, four R lines, 26, 38, 40 and 42 have been classed among the M lines. Likewise, two M lines, 5 and 13, have been classed among the R lines. The line 7 has been located out side the two main clusters. On the dendrogram, one can observe a triplet (lines 1, 2 and 18) and four pairs (lines 3 and 4, lines 29 and 34, lines 37 and 39, and lines 28 and 31) of lines which were very close and had a genetic distance less than 0.05 between them. At morphophysiological level (Table 1), lines 1, 2 and 18 can not be distinct ; likewise for lines 29 and 34. Line 3 differentiated from line 4 only by one character - leaves denture. Lines 37 and 39 shared a common parent; likewise for the pair of lines 28 and 31. However, the last two pairs of lines are declared distinct by morphophysiologic characters.

The results of the principal component analysis were almost the same as those of UPGMA dendrogram (the figure is not showed). The first component separated well the R lines from the M lines, with some exceptions just like UPGMA dendrogram.

Discussion and conclusions

The average number of RFLP variants detected by probe-enzyme combination was about 6; this result confirmed the reports made by Berry et al. (1994), by Gentzbittel et al.(1994) and by Zhang et al. (1995) ; this means that the cultivated sunflower has a relatively high level of RFLP which is comparable with those reported in maize (Messmer et al., 1991, Smith et al; 1991, Livini al et. 1992). This level of polymorphism is three times higher than that revealed by isozymes in sunflower inbred lines (Quillet et al., 1992, Bourgoin-Grenèche and Lallemand, 1993).

The results presented in this study show that the RFLP data have a very good potential in distinctness, identification and description of sunflower inbred lines. The RFLPs have also been proved to be very powerful in establishing the relatedness and in measuring the genetic distance between lines. Compared with morphological characters and isozymic markers, the RFLPs have many advantages as descriptors of lines and varieties: unlimited number, independence to culture conditions, high level of polymorphism and good

reproducibility. This type of descriptors is especially useful for the cultivated sunflower which is extremely susceptible to the climatic conditions; the phenotype of a given sunflower inbred line or a hybrid is often hardly recognized when observed visually in different sites during the same year, and between years. In consequence, the usual morphophysiological characters used for DUS testing in sunflower show more and more limits to discriminate correctly the inbred lines and varieties that increase year by year. In the future, it will proposed that the combination of the use both the classic morphophysiological characters and a genetic distance based on RFLP data could improve largely the accuracy of the distinctness decision on inbred lines and varieties in the DUS testing and strengthen the protection of plant breeder's right.

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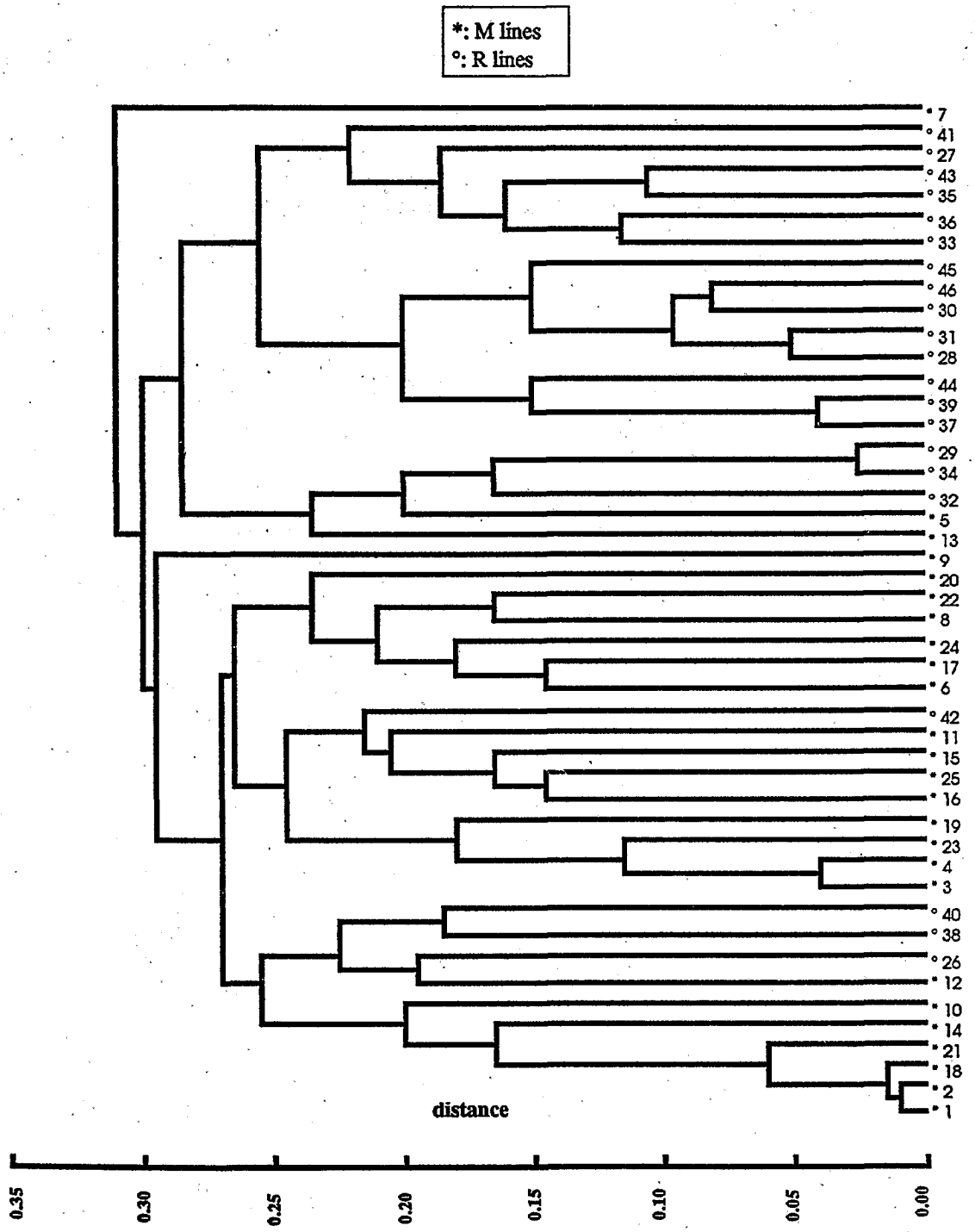

Fig. 1. UPGAM dendrogram generated from Nei's genetic distance estimates showing the relationships among the 46 sunflower inbred lines.

Table 1. RFLP similarity index values and morphological observations for seven pairs of lines which are very close in the field

Pair of lines	Similarity index values of RFLP	Decision of French sunflower DUS experts based on morphophysiological charaters
1, 2	0.98	no distinctness
1, 18	0.98	no distinctness
2, 18	0.97	no distinctness
3, 4	0.93	differentiated only by their leaves teeth
29, 34	0.95	no distinctness
37, 39	0.93	close but distinct (they shared one common parent)
28, 31	0.92	close but distinct (they shared one common parent)