POLYPHENOLIC METABOLISM AS A CHEMICAL FINGER-PRINTING OF SUNFLOWERS.

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SUMMARY

A precise identification of Sunflower lines, as well as the estimation of their homogeneity, are needed for breeding processes. We propose to use a finger-printing based on the phenolic metabolism. We evaluate the stability of this character according to developmental or environmental variations. Within the limits that we define, the comparisons may be done either in identical conditions, or from several cultural assays, so as to reach the stable component of the varietal phenolic pattern.

RESUME

Une identification précise de lignées de Tournesol, ainsi que l'appréciation de leur homogénéité sont nécessaires au sélectionneur. Nous proposons ici d'utiliser un marquage basé sur l'expression du métabolisme phénolique. Nous évaluons la stabilité de ce caractère en fonction des variations liées au développement ou à l'environnement. Dans les limites que nous définissons, les comparaisons peuvent être réalisées soit toutes conditions égales par ailleurs, soit sur plusieurs mises en culture ce qui permet d'atteindre la composante stable de l'expression phénolique variétale.

INTRODUCTION

The first aim of Sunflower breeder is to disclose, within a genetic pool or a segregating line, an originality that might provide a significant gain in the agronomical qualities. Such an originality will be usable so long as the line is distinguishable and homogeneous enough.

Among secondary metabolism, phenolic compounds may be used for that purpose, because:
- being unnecessary to cell life, they have been allowed to develop a large structural diversity, and so provide an accurate and subtle finger-printing;
- it is technically possible to obtain from an individual plant an overview of its phenolic pattern, in which the relative amount of each molecule reflects the expression of structural genes and regulatory genes involved in its biosynthesis;
- those secondary molecules are considered as relational tools and as an evolutive answer of plants to their environment.

It has already been shown (Sanlaville et al., 1988a) (Figure 1) that usual breeding processes have repercussions in the individual phenolic metabolism as a sorting out among the biochemical potentialities of the initial genic pool; thus, 8 independent breeding processes starting from the genic pool HA89 have led to a decrease of phenolic variability of some daughter lines.

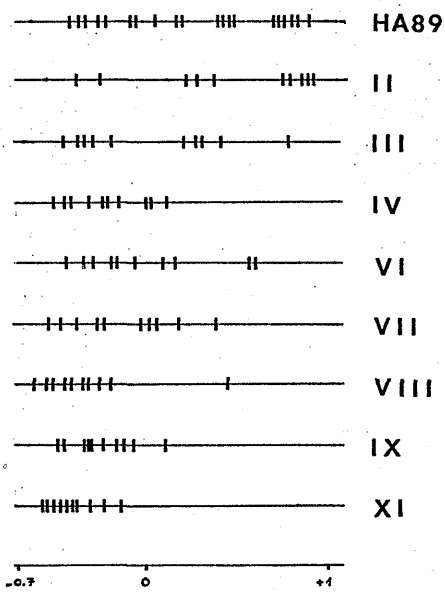

FIGURE 1: FAC treatment of foliar phenolic patterns obtained on a sampling from the genic pool made up of the American line HA89 and 8 lines derived from it by agronomical selection. Reading line by line of the individuals' ordination along axis 1 (inertia: 63%). From Sanlaville et al., 1988a.

If the phenolic metabolism is concerned by breeding processes, it could be used as a finger-printing to evaluate homogeneity, identity and originality of the selected products. In this paper, we will show that the reliability of this tool is not compromised by variations coming from plant development or environment.

MATERIALS AND METHODS

*Plant material:

6 simple and distinct lines: 166A, HA99, HA290, HA400, 11R, RHA801, have been grown up by INRA-GEVES in the following conditions:
- in a greenhouse, for the developmental variation study, at F-78 La Minière in 1986: 4 stages were choosen at blooming time: 4-1, 4-3, 4-5 and 5-0 (according to the CETIOM scale); the harvest concerned a Just Metabolically Mature Leaf (JMML = rank 6-7 from the capitule); additionally, the JMML-2 ranks and the JMML+2 ranks were collected at the 4-3 stage;
- in opened-fields, for the environmental variation study, at: F-78 La Minière in 1984 and 1985, F-17 Le Magneraud in 1985 and F-34 Lavalette in 1985; only the 4-3 stage and the JMML were retained;
5 individual plants were sampled for each line and each experimental condition. Leaves were dried at room temperature.

* Phytochemical analysis:

The plant material was extracted twice with EtOH-H_2O (6-4); the combined extracts were concentrated to dryness under reduced pressure; the residue was extracted with boiling H_2O, and the phenolic fraction transferred into EtOAc; the solvent was evaporated and the residue taken up in a small volume

of MeOH; chromatographic analysis was performed by HPLC on a RP C-18 column (Nucleosil, 5 μ) under UV detection at 328 nm, the mobile phase being a gradient of acetonitrile in H_2O with 2% AcOH; the peaks were marked by retention time and quantified; the data were then translated to % of the sum of total.

*** Mathematical treatment:**

Graphic representations have been made using Auda's software (Auda, 1983); data dealing with environmental variations have been submitted to Discriminant Analysis.

RESULTS

The individual patterns of 6 lines analysed in the same condition (growth, developmental stage and leaf rank) are presented in Figure 2.
The variability within lines is low; comparatively, the variability between lines is greater, allowing easy line distinction.

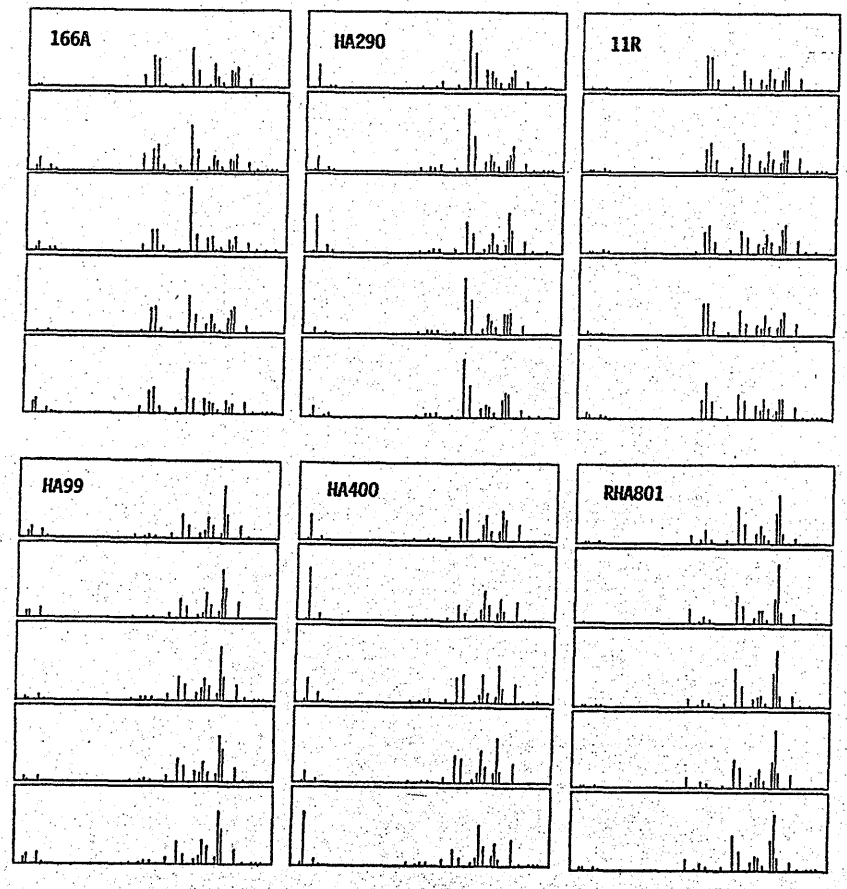

FIGURE 2: Individual foliar phenolic patterns of 6 Sunflower lines grown up in a greenhouse (4-3 stage; Just Metabolically Mature Leaf).

The variations according to the developmental stage, the rank or the growth conditions are shown in Figure 3 for a representative line (HA290).
In comparison with the previous variation within lines, it could be observed that:
- the phenolic pattern seems to undergo noteworthy variations only from the 4-5 stage; these variations are qualitatively different according to the lines, but quantitatively similar;
- the pattern is constant whatever the rank, within the limits of our study;
- greater variations occur between growth conditions; again, they are of different nature according to the lines, but of a comparable level.

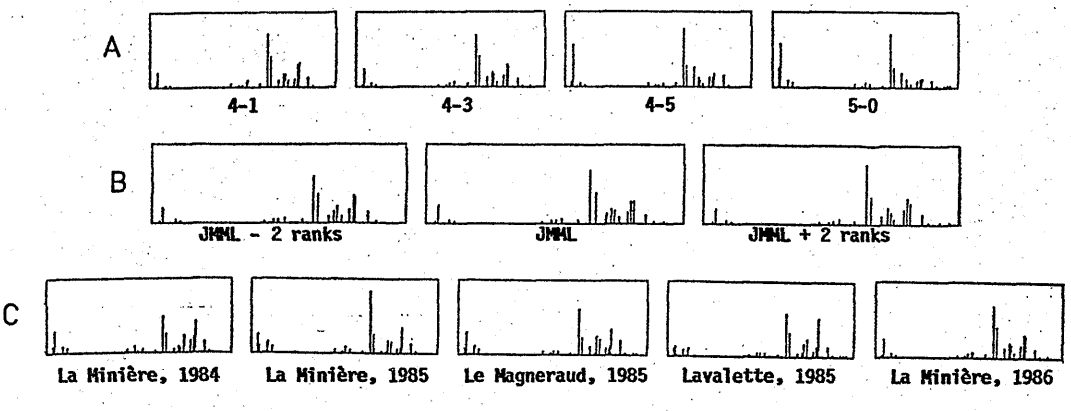

FIGURE 3: Foliar phenolic metabolism variation of line HA290 according to:
- A: the stage (4-1 to 5-0) for the JMML (greenhouse);
- B: the rank (JMML - or + 2 ranks) for the 4-3 stage (greenhouse);
- C: the growing assay for the 4-3 stage and the JMML;
Mean patterns from 5 individuals, for each experimental condition.

DISCUSSION

With respect to plant development, the phenolic pattern remains constant from the 4-1 to the 4-3 stages; the important variation of that pattern from the 4-5 stage could be related to the change from blooming to fructification. The blooming plant makes leaf sampling very flexible between the 4-1 and 4-3 stages and between JMML-2 and JMML+2 ranks.

The Sunflower phenolic metabolism seems to be sensitive to the environmental conditions (edaphic, climatic or meteorologic). But it is possible to distinguish lines from each other by Discriminant Analysis, that provides the stable varietal component of each one: a nearly complete separation between the 6 lines may be obtained by the 1,2 ordination.

Therefore, the finger-printing can be developed into 2 ways:
- from a single culture realized under the same conditions, by direct analysis of plant phenotypes;
- from several growing assays in various environmental conditions, in order to reach a more and more stable varietal component, drawing nearer to the genotypic expression.

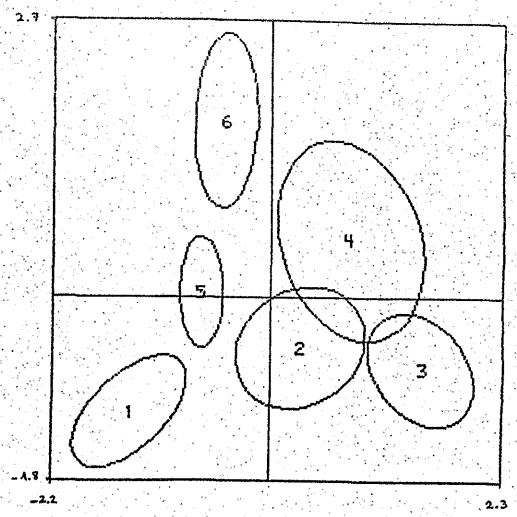

FIGURE 4: Discriminant Analysis (according to the lines) on the foliar phenolic patterns of 6 lines grown up 4 times in opened fields; 1,2 factorial ordination of the individuals; the points corresponding to a same line have been clustered by an ellipse.
Legend: 1: 166A, 2: HA99, 3: HA290, 4: HA400, 5: 11R, 6: RHA801.

CONCLUSIONS

The phenolic finger-printing is thus a reliable and convenient tool. Moreover the biochemical differences between lines seem to reflect differences in breeding processes: the phenolic similarity/dissimilarity levels between lines are comparable with those obtained from a sum of morphological or agronomical characters (Sanlaville et al., 1988b); it appears that this phenolic finger-printing even might constitute alone a synthesis of a whole phenotype. Thus it may be possible, beyond the mere global marking of a selected product, to accede to a printing of interesting agronomical properties and more particularly resistance to pathogens (Hemery et al., 1988).

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