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# DIFFERENTIATION OF *Helianthus* SPECIES BY THIN-LAYER CHROMATOGRAPHY OF LEAF EXTRACTS

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SUMMARY

Leaf extracts from 17 *Helianthus annuus* lines and 18 wild *Helianthus* species were analyzed by thin-layer chromatography with cellulose-coated plates for variation in phenolic compounds. There was no qualitative variation among the cultivated lines. In the wild species, *H. tuberosus* and *H. laciniatus* frequently showed yellow fluorescing components, probably flavonoid compounds, while the two *H. occidentalis* accessions and one *H. mollis* accession were distinguished by a pale blue fluorescing phenolic compound.

**Key words: *Helianthus* species, phenolic compounds, thin-layer chromatography.**

## INTRODUCTION

The phenolic compounds, particularly in the leaves, has often been investigated in various plant species as possible sources of markers or as a tool in grouping the species into various taxa. These compounds have been separated by various chromatographic techniques. In the last few years, HPLC (high performance liquid chromatography) has become popular in separating phenolics and other chemicals extracted from plant leaves. Thus Sanlaville et al. (1988b), reported quantitative variation in the phenolics of 15 sunflower lines from 5 gene pools using HPLC.

Although HPLC is the most sensitive procedure, it is time consuming. Thin-layer chromatography has the advantage of being quick, as several samples can be chromatographed at the same time. This technique was thus investigated as a possible method in differentiating *Helianthus annuus* lines and *Helianthus* species. Preliminary results by this technique were reported earlier (Dedio and Seiler, 1993).

## MATERIALS AND METHODS

Two types of cellulose coating on glass plates, microcrystalline and MN 300 were used. This microcrystalline cellulose-coated plates were chromatographed in water-HCl-propionic acid (10:2:3) in the first direction and in formic-HCl-water (2:1:2) in the second direction (Barritt and Torre, 1975). Cellulose MN 300 plates were chromatographed in 2% formic acid in the first direction and amyl alcohol-acetic acid-water (2:1:1) in the second direction as described by Frost (1966). The spots were detected under UV light either with or without exposure to ammonia or NaOH spray.

Leaf extracts of cultivated *H. annuus* lines and wild *Helianthus* species were used for chromatography. The leaves of 17 cultivated lines from the greenhouse at Morden, MB, and 31 accessions representing 18 wild species were collected in Fargo, N.D., either from the greenhouse or field, and frozen until extraction was carried out. The species and number of accessions (in brackets) were: *Pauciflorus* (=*rigidus*) (2), *tuberosus* (5), *mollis* (2), *grosseserratus* (1), *maximiliani* (2), *salicifolius* (1), *ciliaris* (1), *pumilus* (1), *augustifolius* (1), *nuttallii* (3), *laciniatus* (4), *occidentalis* (2), *giganteus* (1), *microcephalus* (1), *eggertii* (1), *xlaetiflorus* (1), *divaricatus* (1) and *hirsutus* (1).

Two methods of extracting phenolic compounds from leaves were investigated. One was to soak leaf samples overnight in 1% HCl-methanol (10ml for each g of sample) before chromatography. The other method to obtain a purer and more concentrated extract, involved bringing the leaves to boil with methanol only. The next day leaves were removed, small amounts of water added and most of the chlorophyll and lipid constituents removed with hexane. The alcohol-water extract was evaporated almost to dryness and dissolved in methanol (1ml for each g of sample) and chromatographed.

## RESULTS AND DISCUSSION

Good separation of phenolic compounds were obtained with either type of cellulose-coated plates. Single-way chromatography with water-HCl-propionic acid or 2% formic acid yielded 8 or 9 spots. Two-way chromatography usually separated 2 additional spots but they were diffused.

The spots under UV light appeared either as blue fluorescing, a shade of yellow or a dark color.

There were no consistent differences in the chromatograms from leave extracts of the cultivated lines, either hybrids or inbred lines. Minor variation was observed among the inbred lines, which could probably be due to extraction procedure. Quantitative differences among lines may also exist as was reported by Sanlaville et al. (1988a,b).

Among the wild *Helianthus* species, several species could be distinguished by the presence of certain spots (Table 1). The slow moving yellow spots (1 and 3) were characteristic of *H. tuberosus*. *Helianthus laciniatus* showed only the faster moving spot (3) which may or may not be the same as that appearing in *H. tuberosus*. The yellow spots appeared in all *H. tuberosus* and *H. laciniatus* accessions but appeared infrequently in other *Helianthus* species. The slow moving dark spot (2) was present in several species including *H. pauciflorus* (=*rigidus*), *H. grosseserratus, H. maximiliani, H. salicifolius, H. augustifolius* and *H. microcephalus*. A faster moving dark spot (5) was sometimes observed in various species notably, *H. maximiliani* and *H. laciniatus*, but it was not conclusive.

*Helianthus occidentalis* was clearly differentiated from any other species by the presence of a pale blue fluorescing spot (6) which was absent in all other species. A spot similar to and close to 6 was present only in one *H. mollis* accession.

Several blue fluorescing spots (4, 7 and 8) were present in almost all extracts in varying amounts. Spot Number 4 was identified as chlorogenic acid by co-chromatography. This compound is present in the sunflower kernel which produces an undesirable green coloration in the meal in the presence of an alkali. No attempts were made to identify

the other blue or yellow fluorescing spots, but the color of the latter would suggest they are flavonols or flavones.

Table 1. Phenolic compounds detected in 17 *Heliantus* species leaf extracts in thin-layer chromagrams using microcrystalline cellulose plates developed in water-HCl-propionic acid (10:2:3).

| | Spots - Rf - Color | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| | .26 | .35 | .43 | .59 | .65 | .77 | .83 | .91 |
| Species | Yellow | Black | Yellow | Blue | Black | Pale Blue | Blue | Blue |
| *Pauciflorus* | | X | XX | | | XX | X | |
| *Tuberosus* | XX | | XX | XX | | XX | X | |
| *Mollis* | | | | XX | | X | XX | X |
| *Grosseserratus* | X | | XX | | | XX | X | |
| *Maximiliani* | | X | XX | X | | XX | X | |
| *Salicifolius* | X | | XX | | XX | X | | |
| *Ciliaris* | | | | XX | | XX | X | |
| *Pumilus* | | | | XX | | | XX | X |
| *Augustifolius* | | X | | XX | | | XX | X |
| *Nuttallii* | | | | XX | | | XX | X |
| *Laciniatus* | | | X | XX | X | | XX | X |
| *Occidentalis* | | | | XX | | XX | XX | X |
| *Giganteus* | | | | XX | | | XX | X |
| *Microcephalus* | | X | | XX | | | XX | X |
| *Eggertii* | | | | XX | | | XX | X |
| *Xlaetiflorus* | | | | XX | | | XX | X |
| *Divaricatus* | | | | XX | | | XX | X |
| *Hirsutus* | | | | XX | | | XX | X |

XX = Present in large quantities.

X = Present in some quantities or some accessions.

Although this study did not involve all of the *Helianthus* species, examination of the phenolic compounds can confirm the identification of a few species, such as *H. tuberosus*, *H. laciniatus*, and *H. occidentalis*. *Helianthus tuberosus* and *H. laciniatus*, although not related and classified into different sections (Heiser, 1969), had detectable amounts of flavonol compounds. *Helianthus occidentalis* and *H. mollis* on the other hand are morphologically related and are placed in the same series and section, Divaricati. They were the only two species examined with a pale fluorescing spots.

Thin-layer chromatography can be used as a quick method to screen for desirable and undesirable phenolic compounds among species or interspecific hybrids. Quantitative variation, i.e., of chlorogenic acid was observed among species suggesting that it may be possible to select germplasm with reduced levels of the phenolic compound.

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**DIFERENCIACION DE ESPECIES DE *Helianthus* BY CHROMATOGRAFIA DE CAPA FINA DE EXTRACTOS DE HOJA**

RESUMEN:

Extractos de hojas de 17 líneas de *Helianthus annuus* y 18 especies silvestres del género *Helianthus* fueron analizadas, por chromatografia de capa fina con placas revestidas de celuloza, para variación en compuestos fenólicos. No existió variación cuantitativa entre las líneas cultivadas. Las especies silvestres, *H. tuberosus* y *H. laciniatus* mostraron frecuentemente componentes con fluorescencia amarilla, probablemente flavonoides mientras que dos entradas de *H. occidentalis* y una de *H. mollis* mostraron un compuesto fenólico con una fluorescencia azul pálida.

**DIFFÉRENCIATIONS D'ESPÉCES D'*Helianthus* PAR CHROMATOGRAPHIE D'EXTRAITS FOLIAIRES SUR COUCHE MINCE**

RÉSUMÉ:

Les variations en composés phénoliques d'extraits foliaires issus de 17 lignées d'-*Helianthus annuus* et de 18 espéces d'*Helianthus* sauvages ont été analysées par chromatographie en couche mince (support cellulosique). Aucune différence quantative n'a été observée concernant les lignées. Au sein des espéces sauvages, *H. tuberosus* et *H. laciniatus* ont fréquenmment présenté des composés jaunes fluorescents (probablement des composés flavonoiques), tandis que deux populations d'*H. occidentalis* et une population d'*H. mollis* étaient distinguables par un composé phénolique bleu ple.