the unit in about one day's time. The thicknesses used in construction were selected with a mind toward structural rigidity, and should not be lessened if similar materials are used. Small blocks affixed to the spotting platform insure minimal movement of it during use.

Numerous modifications of this device may be fabricated. Small units may be prepared for use with 2 in. wide paper strips. Wider units could handle full-size 18 in. × 22 in. Whatman paper. Spacing of the sample stations may also be adjusted to the preference of the user.

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## Thin layer chromatographic separation of anthocyanins and anthocyanidins in *Medicago* (Papilionaceae)

Paper chromatographic separation of the anthocyanins and anthocyanidins of *Medicago sativa* (Leguminosae, subfamily Papilionaceae), has been reported by several authors[1–3]. LESINS[1] (1956) identified the anthocyanins as derivatives of the three aglycones namely delphinidin, petunidin and malvidin. By means of circular paper chromatography and 5% aqueous orthophosphoric acid as developing solvent he found five anthocyanins which were identified as aglycone delphinidin with two of its derivatives and one derivative each of petunidin and malvidin. In one tetraploid cross he reported presence of only four anthocyanins showing absence of one malvidin derivative. BUKER AND DAVIS (1961)[2] and COOPER AND ELLIOTT (1964)[3] who independently worked with diploid *M. sativa*, on the contrary reported occurrence of only three anthocyanins which were found to be inherited as a single genetic unit without showing any segregation for one malvedin derivative. The latter authors[3] identified the three anthocyanins as 3,5-diglucosides of delphinidin, petunidin and malvidin respectively.

The objective of the current study was to develop a sensitive thin layer chromatographic technique by means of which several accessions of diploid and tetraploid *M. sativa* could be quickly examined and the number of anthocyanins and their aglycones present in this species could be established. A thin layer chromatography (TLC), for the separation of these compounds, has only recently been applied[4,5]. In order to save time from preparing a uniform coating of silica gel, cellulose and other

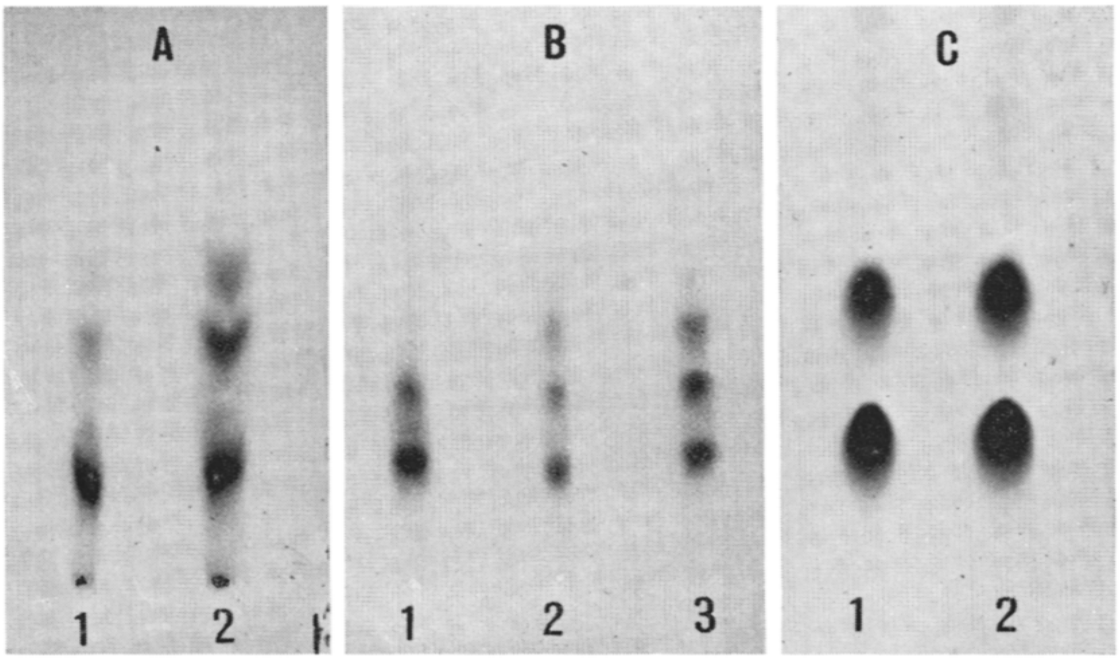


Fig. 1. Separation of anthocyanins and anthocyanidins from flower petals of *M. sativa* and *M. coerulea*. (A) Anthocyanins of *M. sativa* (1, 2—two different accessions) separated on silica gel sheet type 6065 using ethyl acetate–methyl ethyl ketone–formic acid–water (5:3:3:1 v/v) as developing solvent. (B) Anthocyanins of *M. coerulea* (1, 2, 3—three different accessions) separated on cellulose sheet 6064 using BAW (4:1:5 v/v, upper phase) as developing solvent. (C) Two anthocyanidins from the hydrolyzate of two anthocyanins from individual accessions of (1) *M. coerulea* and (2) *M. sativa* respectively separated on cellulose sheet type 6064 using Forestal as developing solvent.

The developing solvents which have been found suitable for the separation of anthocyanidins on paper[7] could also be used for these TLC sheets. In the present study only Forestal (Fig. 1C) and BAW (4:1:5 v/v) were tested and were found very useful for separating the anthocyanidins of different species accessions into two and three different spots.

*Identification.* A single plant, from each of the four different accessions of diploid and two different accessions of tetraploid *M. sativa*, was examined. The plants from two accessions of diploid and one accession of tetraploid *M. sativa* showed presence of only two instead of three or more anthocyanins[1–3] while the rest of the plants from other accessions had three pigments[2,3]. In order to identify the anthocyanins, the acid extract from the flower petals was separated on Whatman No. 1 filter paper using *n*-butanol–acetic acid–water (4:1:5 v/v, upper phase) as the developing solvent. The $R_F$ values for the three components were compared with the values already independently reported by COOPER AND ELLIOTT[3] and HARBORNE[8].

In Table I, the $R_F$ values of the three pigments of *M. sativa* determined by TLC (using silica gel Eastman Chromagram sheet type 6065 and EMFW as developing solvent) and paper chromatography (using Whatman No. 1 filter paper and BAW (4:1:5) as the developing solvent, mean value estimated from eight different chromatograms) are compared. In both paper and thin layer chromatograms (Fig. 1A, plant 1) the anthocyanin farthest from the origin was found missing in case of plants having only two pigments. Consequently on the basis of its highest $R_F$ value (0.30 on paper

the finding of LESINS[1] that some plants of *M. sativa* lacked one anthocyanin—aderivative of malvidin (malvidin 3,5-diglucoside)—and thus loss of malvidin could be inferred. In conclusion it can be summarized that some plants of *M. sativa* contain three anthocyanins in their flowers while others contain only two and the segregating pigment is a derivative of malvidin.

The present technique of thin layer chromatography was found to be simple and less time consuming. A micro amount of pigment mixture could be analysed in less than two hours while it takes 12–14 hours for one dimensional paper chromatography. The TLC sheets could be conveniently stacked in a file for future record.

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1 K. LESINS, *J. Heredity*, 47 (1956) 177.
2 R. J. BUKER AND R. L. DAVIS, *Crop Sci.*, 1 (1961) 437.
3 R. L. COOPER AND F. C. ELLIOTT, *Crop. Sci.*, 4 (1964) 367.
4 N. NYBOM, *Physiol. Plantarum*, 17 (1964) 157.
5 S. ASEN, *J. Chromatog.*, 18 (1965) 602.
6 S. B. GUPTA, *J. Chromatog.*, 36 (1968) 258.
7 J. B. HARBORNE, in M. LEDERER (Editor), *Chromatographic Reviews*, Vol. 1, Elsevier, Amsterdam, 1959, pp. 209–224.
8 J. B. HARBORNE, *J. Chromatog.*, 1 (1958) 473.

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