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# Disc electrophoretic study of seed proteins of various *Medicago* species, *Melilotus albus*, *Trifolium pratense*, and *T. repens*

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Abstract:

Disc electrophoretic study covered albumins, globulins and urea-treated globulins of seeds of 12 *Medicago* species, *Melilotus albus*, *Trifolium pratense* and *T. repens*. The electrophoretic data differentiated most of the examined *Medicago* species and indicated *M. lupulina* to be especially distinct. Some similarities of *M. lupulina* to *Melilotus* and *Trifolium* have been pointed out.

## INTRODUCTION

Data provided by biochemical and chemical studies resulted in an increased interest in chemotaxonomic investigations. The employment of different chemical data in systematic studies is being largely stimulated by rapid development of relatively quick and simple analytical procedures. Also, the emphasis given recently to proteins as taxonomic characters is partly a consequence of developing the appropriate methods of analysis.

Disc electrophoresis belongs to the methods widely employed nowadays to compare the properties of proteins (see reviews: Steward, Barber 1964; Boulter, Thurman, Turner 1966; Boulter, Thurman 1968; Fairbrothers 1968, 1969, Johnson 1969). As pointed out by Boulter and Thurman (1968), "the method is highly feasible technically in its application in systematic surveys since it is quick, simple and inexpensive to use".

The subject of the present study is to compare disc electrophoretic patterns of seed proteins of chosen *Medicago* species and of the species representing related genera *Melilotus* and *Trifolium*. The investigations intend to check whether this approach may be useful in attacking taxonomic problems of the genus *Medicago*. There are controversial opinions concerning classification of this group which comprises about fifty species largely differing in morphology and geographical distribution (Sinskaja 1950).

The electrophoretic analysis covers albumins, globulins and urea-treated globulins. Albumins were electrophorized in basic buffer system only, while untreated

List of the species analysed [a/]

Table 1

| Genus and subgenus | Section or series | Species | Origin | Year of harvest | Seed source [b/] |
|---|---|---|---|---|---|
| Medicago L.: | | | | | |
| Lupularia /Ser./ Grossh. | | M.lupulina L. | Białystok region, Poland | 1967 | G |
| | | - " - | Kraków region, Poland | 1967 | C |
| Falcago /Rchb./ Grossh. | Brachycarpa Grossh. | M.falcata L./Krasnokutskaja 4009/ | Saratov region, USSR | | A |
| | | - " - /Stepnaja 600/ | Voronezh region, USSR | | A |
| | | M.quasifalcata L./Kubanskaja mestnaja/ | Krasnodar region, USSR | | A |
| | | - " - Sinsk./wild-growing/ | Krasnodar region, USSR | | A |
| | Vulgares Grossh. | M.media Pers. /Grimma/ | Warsaw region, Poland | 1965 | F |
| | | - " - /Puławska/ | Lublin region, Poland | 1965 | E |
| | | - " - /Vernal/ | Idaho, USA | 1965 | B |
| | | M.sativa L. /Taškentskaja 3192/ | Uzbekistan, USSR | | A |
| | | - " - /Uzgentskaja mestnaja/ | Kirghizia, USSR | | A |
| | | - " - /African/ | Israel | 1967 | C |
| | | M.coerulea Less. | Daghestan, USSR | | A |
| | Hemicyclae Grossh. | M.tianschanica Vass./wild-growing/ | Kazakhstan, USSR | | A |
| | | M.hemicycla Grossh. | Armenia, USSR | | A |
| | | - " - | Georgia, USSR | | A |
| | Glutinosae Grossh. | M.polychroa Grossh./wild-growing/ | Stavropol region, USSR | | A |
| Orbicularia Grossh. | Scutellatae Urb. | M.scutellata All. | Israel | 1967 | C |
| | Orbiculares Urb. | M.orbicularis All. | Israel | 1967 | C |
| Spirocarpos /Ser./Grossh. | Euspirocarpae Urb. | M.denticulata Willd. | Israel | 1967 | C |
| Melilotus Adans.: | | | | | |
| | | M.albus Desr. /Arktyk/ | Gorzów Wlkp., Poland | 1964,1969 | D |
| | | - " - /Małyszyński/ | Gorzów Wlkp., Poland | 1964,1969 | D |
| Trifolium L.: | | | | | |
| | | T.pratense L. /Dollard/ | California, USA | 1965 | B |
| | | - " - /Hruszowska/ | Lublin region, Poland | 1965 | F |
| | | T.repens L. /Common/ | Idaho, USA | 1965 | B |
| | | - " - /Podkowa/ | Kielce region, Poland | 1965 | F |

a/ The Medicago species are arranged according to the classification system of Grossheim /1945/. Variety information, if available, is given in parantheses.

b/ Key to the seed sources is given in the text.

and urea-treated globulins were subjected to electrophoresis in both basic and acidic systems. Such a complex analysis, as most informative, has been suggested by results of the previous methodical studies covering several *Medicago* species (Przybylska, Hurich 1971).

## MATERIAL AND METHODS

### Plant material

The object of studies consisted of seeds of 12 *Medicago* species, 2 *Trifolium* species and of *Melilotus albus*. Some of the examined species were represented by seed samples originating from different sources and/or from different years of harvest. In total 28 seed samples were analysed; the samples are listed in table 1.

Following are the seed sources:

A — All-Union Institute of Plant Industry, Leningrad, USSR.
B — U.S. Department of Agriculture, ARS, New Crops Research Branch, Washington, USA.
C — Institute of Plant Breeding and Acclimatization, Radzików near Warsaw, Poland.
D — Institute of Soil Science and Cultivation of Plants, Gorzów Wlkp., Poland.
E — Institute of Soil Science and Cultivation of Plants, Puławy, Poland.
F — Plant Breeding Stations, Poland.
G — "Centrala Nasienna" (Seed Association), Poznań, Poland.

No attempt was made by the authors to check the identity of seed samples.

### Analytical procedures

Electrophoretic analysis covered albumins, globulins and urea-treated globulins. The protein fractions were obtained as described in the previous paper (Przybylska, Hurich 1971).

Electrophoresis of proteins was performed on polyacrylamide gel. All protein fractions examined were electrophorized in tris-glycine buffer pH 8.3 (Davis 1964), while untreated and urea-treated globulins were likewise subjected to electrophoresis in acetic acid-β-alanine buffer pH 4.5 (Reisfeld, Lewis, Williams 1962). Gels were stained with aniline blue black.

Protein contents in the extracts were determined by the method of Lowry et al. (1951), as modified by Miller (1959).

Detailed description of the procedure has been presented in the previous paper (Przybylska, Hurich 1971).

### Interpretation and presentation of results

$R_p$ value of individual band on each diagram represents mean $R_p$ value calculated from six similar gels obtained from two extractions and three electrophoretic runs. Selected protein patterns have been also recorded photographically and by means

of densitometric tracings, electrophoretic spectra being adjusted to equivalent migration velocities. The densitometric tracings were made with a Vitatron-densitometer, type No UFD 500; a filter with a maximum transmittance at 576 mμ was used.

Percentage similarity between two taxa was calculated according to Whitney et al. (1968) as:

$$\frac{\text{No. of pairs of similar bands}}{\text{No. of different bands} + \text{No. of pairs of similar bands}}$$

Percentage similarities were calculated for albumins and for globulins. In case of globulins, both basic and acidic gels were used for the calculations.

## RESULTS

To check whether protein patterns of the taxa investigated vary with source of the material or year of harvest different seed samples were analysed for some of the examined species (see Table 1). In no case samples of the same species differed in respect to electrophoretic protein patterns though they varied in the total protein level.

While comparing electrophoretic patterns of seed proteins of the examined *Medicago* species it was observed that the corresponding spectra of *M. media*, *M. sativa*, *M. hemicycla* and *M. polychroa* were indistinguishable. Protein patterns of *M. sativa*, as of a representative of the above mentioned species, are shown in form of diagrams (see Fig. 1 and 2) and in form of photographs and densitometric tracings of the gels (see Fig. 3 and 4). Similar protein patterns were afforded by *M. falcata*, *M. quasifalcata*, *M. tianschanica* and *M. coerulea*. Concerning qualitative differences, *M. falcata* was distinguished by one additional globulin band revealed in basic buffer system ($R_p$ 0.81), while, in the same system, *M. quasifalcata* exhibited two additional globulin bands ($R_p$ 0.71; 0.81) (see Fig. 1). *M. tianschanica* differed from *M. sativa* by the lack of one band on basic gels of albumins and of urea-treated globulins — protein band at $R_p$ 0.81 was not detected in any of these fractions.

*Medicago scutellata*, *M. orbicularis* and *M. denticulata* varied significantly in their protein patterns which differed also remarkably from those of the above eight species. The qualitative and quantitative differences could be observed in albumin and in globulin fractions. Regarding globulins, essential differences were likewise revealed after urea-treatment (see Fig. 1 and 2).

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Fig. 1. Diagrams of basic gel patterns of albumins (A), globulins (G), and urea-treated globulins (GU) from seeds of various *Medicago* species, *Melilotus albus*, *Trifolium pratense*, and *T. repens*. Abbreviations for taxa: ML, *Medicago lupulina*; MF, *M. falcata*; MQ, *M. quasifalcata*; MS, *M. sativa*; MT, *M. tianschanica*; MSc, *M. scutellata*; MO, *M. orbicularis*; MD, *M. denticulata*, MeA, *Melilotus albus*; TP, *Trifolium pratense*; TR, *T. repens*. Legend as for Fig. 2.

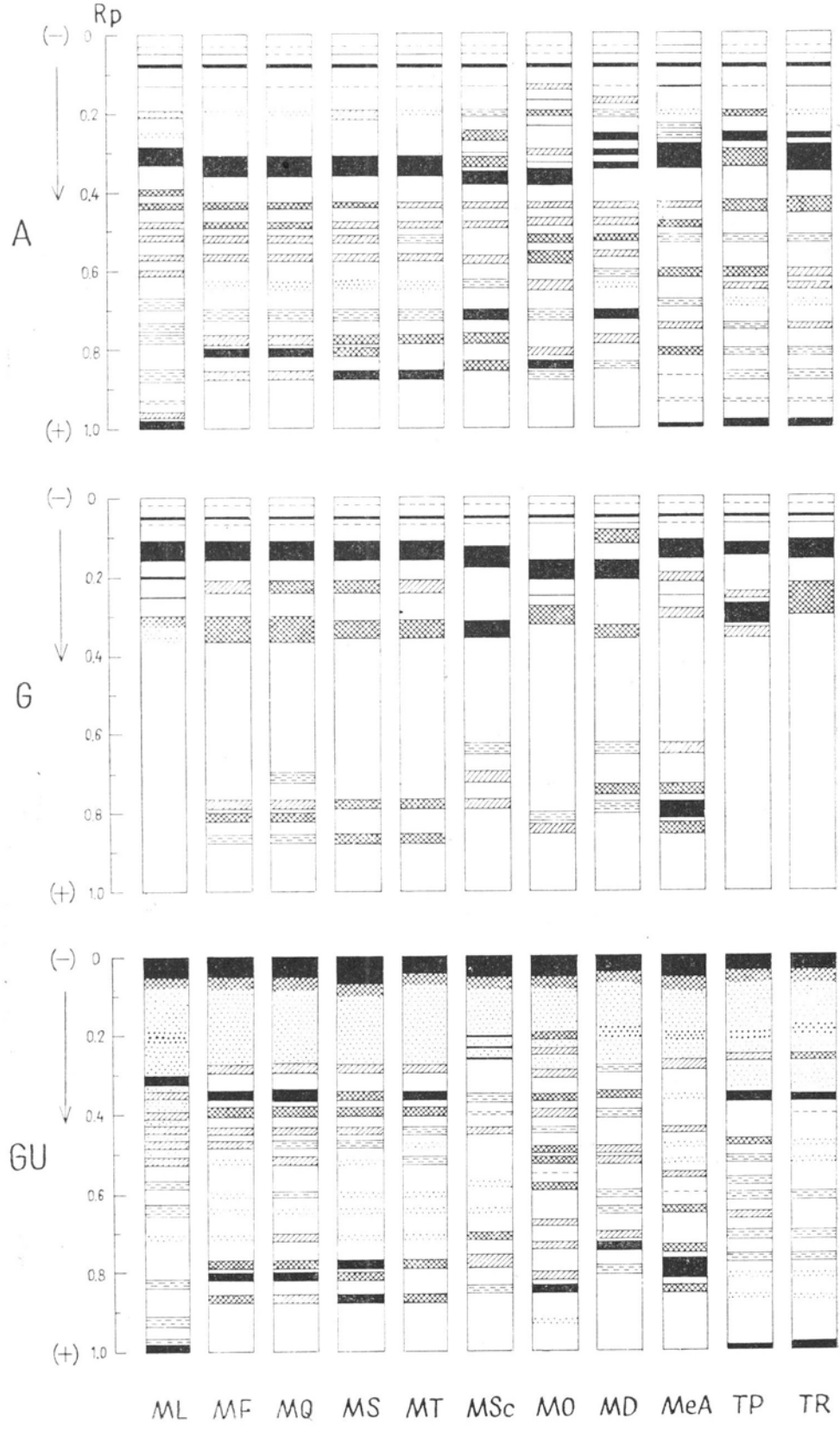


Fig. 1

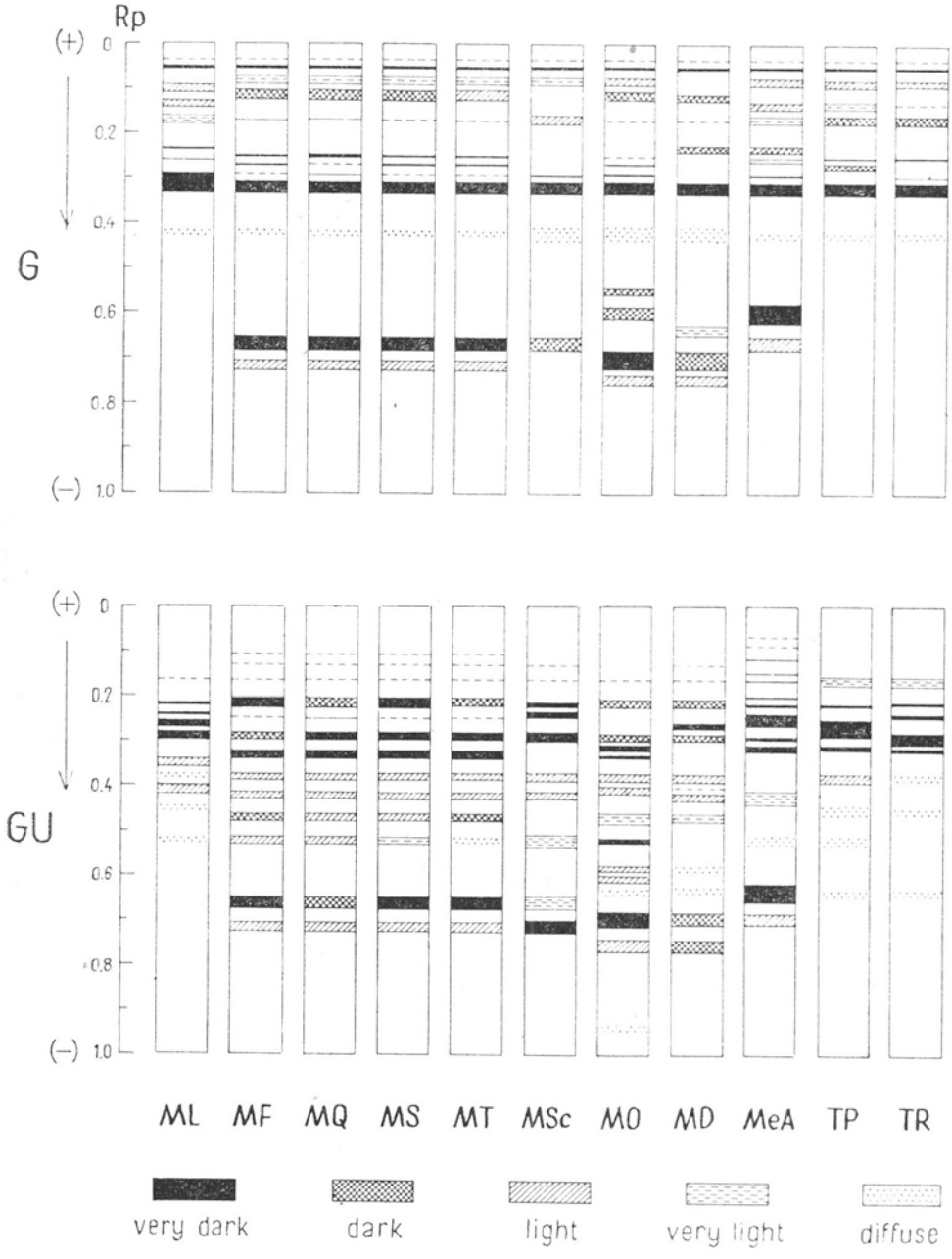


Fig. 2. Diagrams of acidic gel patterns of globulins (G) and urea-treated globulins (GU) from seeds of various *Medicago* species, *Melilotus albus*, *Trifolium pratense* and *T. repens*.

Abbreviations for taxa are given in fig. 1

*Medicago lupulina* showed to be especially distinguished from among the examined *Medicago* species. The differences in electrophoretic patterns could be observed in every protein fraction analysed which is illustrated in figures 1 and 2. Albumin gels revealed more bands, fast-moving ones ($R_p$ 0.93; 0.97; 1.0) being especially characteristic of the species. As regards globulins, bands of high $R_p$ values — observed in all the remaining *Medicago* species under study — have not been detected either in basic or in acidic buffer system (see Fig. 1 and 2). Moreover, electrophoretic patterns of urea-treated globulins of *M. lupulina* were qualitatively different — independently on the buffer system applied — from corresponding patterns of other *Medicago* species examined (see Fig. 1 and 2). Seed protein patterns of *M. lupulina*, as compared with those of *M. sativa*, are presented in figures 3 and 4 in form of photographs and densitometric tracings of the gels.

The analysed *Trifolium* species, *T. pratense* and *T. repens*, differed qualitatively one from another in globulin fraction only, the differences being observed in both buffer systems applied (see Fig. 1 and 2).

When comparing overall protein patterns of the analysed *Medicago* species and those of *Melilotus albus*, *Trifolium pratense* and *T. repens*, it may be observed that differences at the generic level do not exceed variations at the level of species (see Fig. 1 and 2). Moreover, it may be noticed that *M. lupulina* is more closely related — in respect to electrophoretic patterns of seed proteins — to *Melilotus* and *Trifolium* than to other *Medicago* species under study. Two of the fast-moving albumin bands characteristic of *M. lupulina* ($R_p$ 0.93; 1.0) were found in *Melilotus* and *Trifolium* while lack of fast-moving globulin bands was a common feature of *M. lupulina* and *Trifolium*. Electrophoretic patterns of urea-treated globulins of *M. lupulina* were more similar to corresponding patterns of *Trifolium* species and of *Melilotus albus* than to those provided by other *Medicago* species examined.

The relationships among the investigated taxa, expressed as percentage similarities between seed albumins and globulins, are shown in figures 5 and 6. The values provide another kind of illustration of the above presented similarities and differences among the analysed species.

Identity of seed protein patterns of *Medicago sativa*, *M. media*, *M. polychroa* and *M. hemicycla* is reflected in 100 per cent similarity between seed albumins and globulins of any two species of this group. Also, considerable similarity of protein spectra of the above species with those of *M. falcata*, *M. quasifalcata*, *M. tianschanica* and *M. coerulea* is well illustrated by high percentage of similarities which, depending on the protein fraction analysed, range from 80 to 100 per cent.

The percentage similarity between seed albumins and globulins of any one of the above mentioned *Medicago* species and corresponding fractions of *M. scutellata*, *M. orbicularis* or *M. denticulata* proved to be relatively low. The values reported show likewise seed protein patterns of *M. scutellata*, *M. orbicularis* and *M. denticulata* to be different. As globulins were electrophorized in basic and acidic buffers, it could be observed that values expressing percentage similarities varied depending on the buffer system applied.

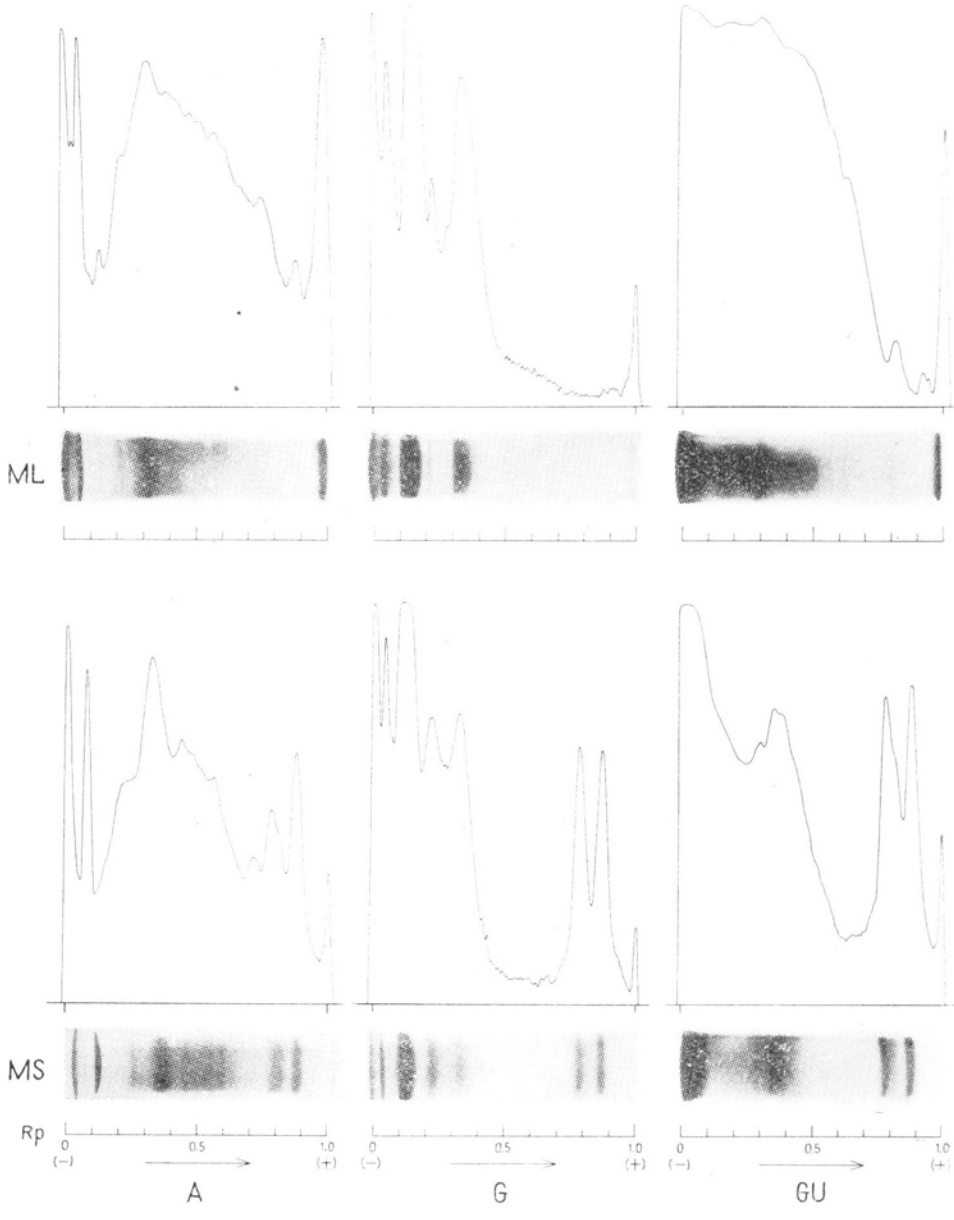


Fig. 3. Photographs and densitometric tracings of basic gels of albumins (A), globulins (G), and urea-treated globulins (GU) from seeds of *Medicago lupulina* (ML) and *M. sativa* (MS).

Fig. 4. Photographs and densitometric tracings of acidic gels of globulins (G) and urea-treated globulins (GU) from seeds of *Medicago lupulina* (ML) and *M. sativa* (MS).

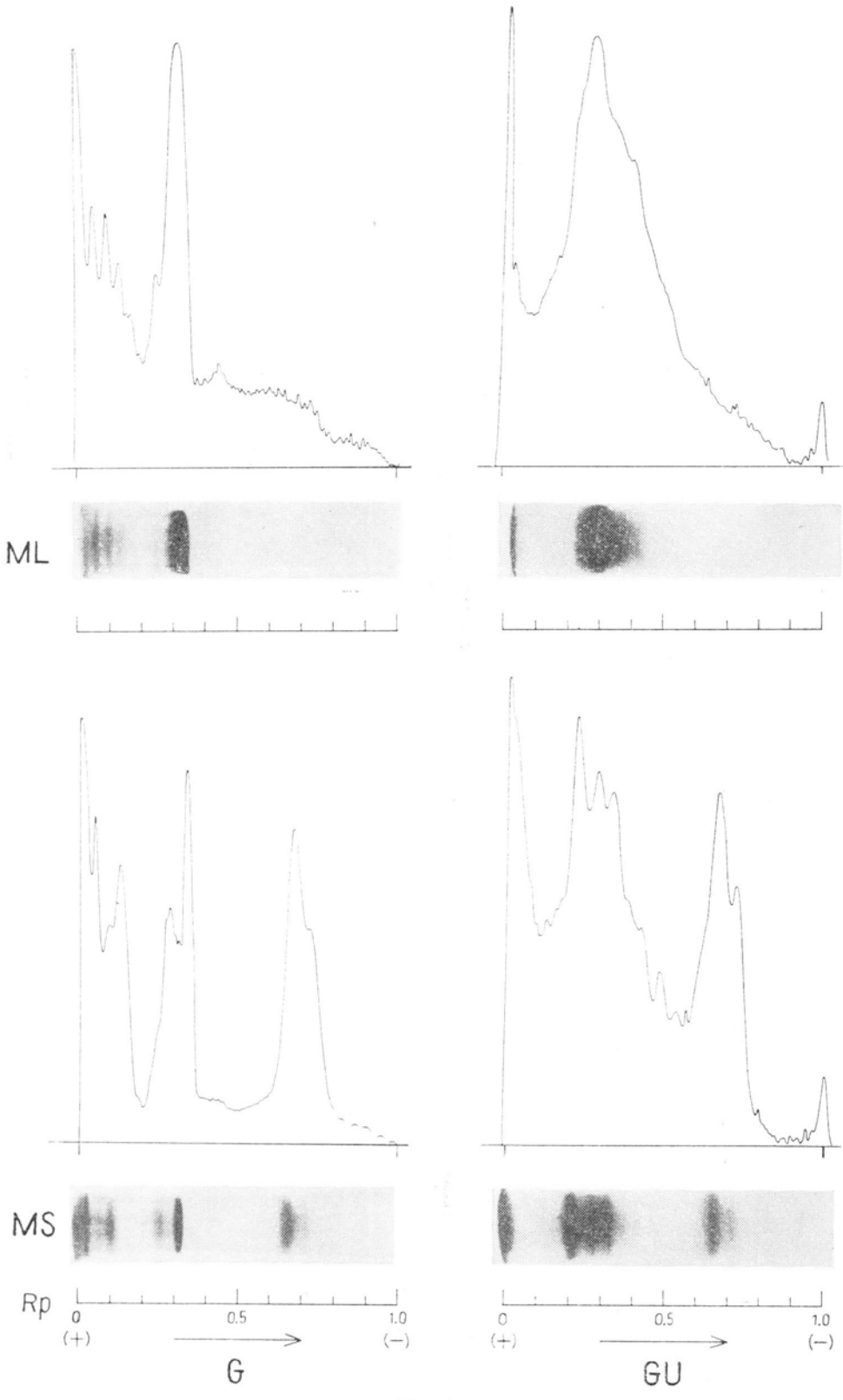


Fig. 4

ALBUMINS

| | MF | MQ | MM | MS | MC | MT | MH | MP | MSc | MO | MD | MeA | TP | TR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ML | 52 | 52 | 52 | 52 | 55 | 55 | 52 | 52 | 44 | 35 | 52 | 73 | 68 | 68 |
| MF | 100 | 100 | 100 | 93 | 93 | 100 | 100 | 63 | 62 | 65 | 50 | 52 | 52 | |
| MQ | 100 | 100 | 93 | 93 | 100 | 100 | 63 | 62 | 65 | 50 | 52 | 52 | | |
| MM | 100 | 93 | 93 | 100 | 100 | 63 | 62 | 65 | 50 | 52 | 52 | | | |
| MS | 93 | 93 | 100 | 100 | 63 | 62 | 65 | 50 | 52 | 52 | | | | |
| MC | 100 | 93 | 93 | 67 | 57 | 68 | 45 | 48 | 48 | | | | | |
| MT | 93 | 93 | 67 | 57 | 68 | 45 | 48 | 48 | | | | | | |
| MH | 100 | 63 | 62 | 65 | 50 | 52 | 52 | | | | | | | |
| MP | 63 | 62 | 65 | 50 | 52 | 52 | | | | | | | | |
| MSc | 67 | 79 | 36 | 38 | 38 | | | | | | | | | |
| MO | 68 | 48 | 44 | 44 | | | | | | | | | | |
| MD | 44 | 46 | 46 | | | | | | | | | | | |
| MeA | 84 | 84 | | | | | | | | | | | | |
| TP | 100 | | | | | | | | | | | | | |

GLOBULINS

| | MF | MQ | MM | MS | MC | MT | MH | MP | MSc | MO | MD | MeA | TP | TR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ML | 45 | 42 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 36 | 33 | 50 | 75 | 50 |
| MF | 90 | 89 | 89 | 89 | 89 | 89 | 89 | 55 | 31 | 38 | 33 | 45 | 40 | |
| MQ | 80 | 80 | 80 | 80 | 80 | 80 | 64 | 29 | 36 | 31 | 42 | 36 | | |
| MM | 100 | 100 | 100 | 100 | 100 | 60 | 23 | 42 | 36 | 50 | 44 | | | |
| MS | 100 | 100 | 100 | 100 | 60 | 23 | 42 | 36 | 50 | 44 | | | | |
| MC | 100 | 100 | 100 | 60 | 23 | 42 | 36 | 50 | 44 | | | | | |
| MT | 100 | 100 | 60 | 23 | 42 | 36 | 50 | 44 | | | | | | |
| MH | 100 | 60 | 23 | 42 | 36 | 50 | 44 | | | | | | | |
| MP | 60 | 23 | 42 | 36 | 50 | 44 | | | | | | | | |
| MSc | 23 | 55 | 46 | 50 | 44 | | | | | | | | | |
| MO | 29 | 46 | 50 | 30 | | | | | | | | | | |
| MD | 43 | 33 | 27 | | | | | | | | | | | |
| MeA | 50 | 33 | | | | | | | | | | | | |
| TP | 71 | | | | | | | | | | | | | |

Fig. 5. Percentage similarities among seed albumins and globulins of *Medicago* species, *Melilotus albus*, *Trifolium pratense* and *T. repens* — based on the basic gel patterns.

Abbreviations for taxa are given in Fig. 6.

GLOBULINS

| | MF | MQ | MM | MS | MC | MT | MH | MP | MSc | MO | MD | MeA | TP | TR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ML | 31 | 31 | 31 | 31 | 31 | 31 | 31 | 31 | 42 | 28 | 46 | 57 | 50 | 50 |
| MF | | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 67 | 73 | 47 | 56 | 62 | 62 |
| MQ | | | 100 | 100 | 100 | 100 | 100 | 100 | 67 | 73 | 47 | 56 | 62 | 62 |
| MM | | | | 100 | 100 | 100 | 100 | 100 | 67 | 73 | 47 | 56 | 62 | 62 |
| MS | | | | | 100 | 100 | 100 | 100 | 67 | 73 | 47 | 56 | 62 | 62 |
| MC | | | | | | 100 | 100 | 100 | 67 | 73 | 47 | 56 | 62 | 62 |
| MT | | | | | | | 100 | 100 | 67 | 73 | 47 | 56 | 62 | 62 |
| MH | | | | | | | | 100 | 67 | 73 | 47 | 56 | 62 | 62 |
| MP | | | | | | | | | 67 | 73 | 47 | 56 | 62 | 62 |
| MSc | | | | | | | | | | 47 | 38 | 62 | 70 | 55 |
| MO | | | | | | | | | | | 50 | 50 | 53 | 53 |
| MD | | | | | | | | | | | | 35 | 36 | 36 |
| MeA | | | | | | | | | | | | | 57 | 69 |
| TP | | | | | | | | | | | | | | 80 |

Fig. 6. Percentage similarities among seed globulins of *Medicago* species, *Melilotus albus*, *Trifolium pratense* and *T. repens* — based on the acidic gel patterns.

Abbreviations for taxa: ML, *Medicago lupulina*; MF, *M. falcata*; MQ, *M. quasifalcata*; MM, *M. media*; MS, *M. sativa*; MC, *M. coerulea*; MT, *M. tianschanica*; MH, *M. hemicycla*; MP, *M. polychroa*; MSc, *M. scutellata*; MO, *M. orbicularis*; MD, *M. denticulata*; MeA, *Melilotus albus*; TP, *Trifolium pratense*; TR, *T. repens*.

Low percentage similarity between *M. lupulina* and any other *Medicago* species analysed was found in case of both albumin and globulin fractions. Higher percentage similarities were recorded between protein patterns of *M. lupulina* on the one hand and those of *Melilotus* and *Trifolium* on the other.

## DISCUSSION

Differentiation of the taxa investigated on the basis of electrophoretic patterns of seed proteins is in general consistent with that based on morphological features, i.e. with the existing classification presented in the Sinskaja's monograph of the genus (1950).

Present studies have indicated that *Medicago* species representing subgenus *Falcago*, namely *M. falcata*, *M. quasifalcata*, *M. media*, *M. sativa*, *M. coerulea*, *M. tianschanica*, *M. hemicycla* and *M. polychroa*, are similar or identical in respect

to electrophoretic patterns of seed proteins. Such a situation could be expected, as subgenus *Falcago* comprises closely related perennial species among which intercrossing is rather commonly observed. The hybrid forms are greatly responsible for the lack of clear-cut morphological differences.

The examined annual *Medicago* species, *M. orbicularis*, *M. scutellata* and *M. denticulata*, afforded different protein patterns which is a good reflection of morphological differences among these species. *M. orbicularis* and *M. scutellata*, though belonging to the same subgenus *Orbicularia*, have been included in separate sections, distinct both in respect to morphological and physiological features, *Orbiculares* and *Scutellatae*, respectively. *M. denticulata* represents a separate subgenus — *Spirocarpos*.

Results provided by gel electrophoresis of seed proteins indicated *Medicago lupulina* of subgenus *Lupularia* to be especially distinguished out of the examined *Medicago* species. In this connection unique taxonomic position of the subgenus *Lupularia* should be born in mind. The subgenus, comprising two species: *M. lupulina* and *M. secundiflora*, is morphologically so distinct from other members of *Medicago* that its placement within this genus has been repeatedly questioned by systematists (Sinskaja 1950; Heyn 1963).

The resemblance of protein patterns of *Medicago lupulina* to those of *Melilotus* and *Trifolium* fits to the opinions based on morphological features that subgenus *Lupularia* is more closely related to the above mentioned genera than other members of the genus *Medicago*.

Because of great variability in morphological and physiological features within *Medicago*, the genus has been suggested to be divided into two or more groups forming separate genera. Such a situation explains, to some extent, the remarkable differences in protein patterns among *Medicago* species.

Some of the results of the present investigations confirm the other authors' data coming from disc electrophoretic studies of seed proteins. Boulter and coworkers (1967) reported fast-moving globulin bands — undetected in *Trifolium* species — to be characteristic of *Medicago* and *Melilotus*. This finding is essentially consistent with presently reported results. However, in the present studies, covering wider range of *Medicago* species, it was possible to show that *M. lupulina* is distinguished from among *Medicago* species by the lack of fast-moving globulin bands.

The distinct properties of seed proteins of *Medicago lupulina* have been likewise indicated by serological studies of Simon (1969). In result of these studies, covering 34 *Medicago* species, most of the examined species were found to be serologically homogeneous. It should be pointed out, however, that *M. lupulina* and *M. secundiflora* were distinctive. Moreover, serological data indicated remarkable affinity of *M. lupulina* to *Melilotus*. In consequence of these observations Simon has proposed reexamination of taxonomic position of the subgenus *Lupularia*. The proposal is supported by presently reported results obtained from a different approach to comparative studies of proteins.

The reported results provide further evidence that seed proteins may serve as plant characters of value for taxonomy. Electrophoretic patterns obtained showed to be constant, irrespective of the seed source, and provided basis for differentiation of the examined taxa which correlated in general with existing classification.

Species represented the lowest compared taxa in the present investigations as preliminary studies of several cultivated varieties of *Medicago* media did not show any differentiation. The lack of intervarietal differences might result from hybrid origin of seeds. It should be mentioned that inbred lines of *Medicago media* var. Vernal proved to be different in respect to albumin patterns (unpublished results).*

Differentiation of the investigated taxa was possible due to analysing different protein fractions and employing two buffer systems for electrophoretic separation of proteins. The advantage of such a broadened electrophoretic analysis has been already pointed out in the previous paper (Przybylska, Hurich 1971) and is confirmed by the present studies covering wider plant material. Qualitative differences between some of the examined taxa would not have been detected if both albumin and globulin fractions had not been analysed and two buffer systems applied. Electrophoretic analysis of urea-treated globulins yielded supplementary information on this fraction.

The literature data show that, depending on the plant material analysed, albumin or globulin fraction of seed proteins proves to be more informative in comparative studies. Gel electrophoretic studies of the seed proteins of *Brassica* and *Sinapis* species had shown that albumin patterns gave a better correlation with the established classification (Vaughan, Denford 1968). On the other hand, investigations carried out with numerous members of *Leguminosae* have indicated taxonomic value of globulin band patterns (Boulter, Thurman, Derbyshire 1967). In the present studies no preference of albumin or globulin patterns as taxonomic characters could be demonstrated, for the fractions supplied complementary data.

## SUMMARY

Twelve *Medicago* species, *Melilotus albus*, *Trifolium pratense* and *T. repens* have been compared in respect to disc electrophoretic patterns of seed proteins. Some of the examined species were represented by seed samples originating from different sources. In total 28 seed samples were examined. The electrophoretic analysis covered albumins, globulins and urea-treated globulins. Albumins were electrophorized in basic buffer system, while untreated and urea-treated globulins were subjected to electrophoresis in both basic and acidic systems.

The electrophoretic data differentiated most of the examined *Medicago* species and indicated *M. lupulina* to be especially distinct. Some similarities of *M. lupulina* to *Melilotus* and *Trifolium* have been pointed out. Seed samples of individual species from different sources proved to be identical in respect to electrophoretic protein patterns.

The results obtained have been discussed with reference to the established classification. There have been also considered some methodical aspects of the studies.

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## *Analiza elektroforetyczna białek nasion różnych gatunków Medicago, Melilotus albus, Trifolium pratense i T. repens przy pomocy elektroforezy na żelu poliakrylamidowym*

Streszczenie

W oparciu o elektroforetyczne obrazy białek nasion porównano dwanaście gatunków *Medicago*, *Melilotus albus*, *Trifolium pratense* i *T. repens*. Dla niektórych gatunków analizowano próby nasion pochodzące z różnych źródeł. Ogółem zanalizowano 28 prób. Elektroforezę przeprowadzano na żelu poliakrylamidowym. Analiza elektroforetyczna obejmowała albuminy, globuliny i globuliny traktowane mocznikiem. Albuminy rozdzielano elektroforetycznie w zasadowym układzie buforowym. Globuliny, nietraktowane oraz traktowane mocznikiem, poddawano elektroforezie zarówno w układzie zasadowym, jak i kwaśnym.

Dane elektroforetyczne wykazały zróżnicowanie większości badanych gatunków *Medicago* ujawniając szczególną odrębność *M. lupulina*. Zaobserwowano pewne podobieństwa obrazów białkowych *M. lupulina* w stosunku do obrazów *Melilotus* i *Trifolium*. Próby nasion poszczególnych gatunków pochodzące z różnych źródeł nie wykazały różnic w elektroforetycznych obrazach białkowych.

Uzyskane wyniki omówiono w nawiązaniu do stanowiska systematycznego badanych gatunków. W dyskusji uwzględniono również metodyczny aspekt badań.