INSTITUTUM AGRONOMICUM "DR. PETRU GROZA" CLUJ-NAPOCA (ROMANIA)
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MICROEVOLUTION IN TRIFOLIUM L. SECT. STENOSTOMA M. B.
I. BIOMETRY OF SPONTANEOUS AND CULTIVATED TRIFOLIUM PANNONICUM JACQ.

A. T. SZABÓ

Abstract

SZABÓ T.A., 1987, Microevolution in Trifolium L. Sect. Stenostoma M.B. I. Biometry of spontaneous and cultivated Trifolium pannonicum Jacq. Not. bot. hort. agrobot., Cluj.,XVII,47-75.
The variability of spontaneous and cultivated (selected) Trifolium pannonicum Jacq. (2n = 16x - 22x !) has been studied by using herbal specimens, samples collected from spontaneous populations, cultivated ones and selected elite plants. The biometry of economically and/or taxonomically important characters have been performed, results tabulated and statistical indicators - mean, standard deviation, coefficient of variation - calculated. These values indicate differences between the eastern and western accessions with regard to peduncule length, petiole length, leaf size, hairiness etc. Based on these differences a new taxon var. moldavicum var. nova has been proposed.

Cultivation revealed, that the diploid T. ochroleucon Huds. is a short-living plant (1-2 years), but polyploid T. pannonicum persisted up to 10 years or more. Natural selection for disease resistence and persistence did not narrowed sharply the variablity of the selected elites. In elite plants the coefficient of variability was especially high for underground organs (number of lateral roots, root nodule for unit root length etc.), characters positively correlated with yield components.

Problems relating to taxonomy, evolution, domestication and use of the species are also briefly discussed.

Key words: Trifolium, taxonomy, biometry, evolution, domestication.

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Introduction

Polyploidization in Trifolium always fascinated breeders (19,45). It is quite clear that polyploidy was connected with clover evolution well before man used it for his own purposes. The peak of the process represents the species T. pannonicum Jacq. - considered subendemic for the Carpathian Basin. This giant clover is clearly preadapted in many treats for domestication, but cultivar release has been held up for various reasons. Despite former failures of cultivation, the species still remains a promising genetic resource as a companion legume for polyploid forage grasses, such as e.g. Festuca arundinacea L., for ecological reconstruction, revegetation of spoil banks, forage for the wild etc., having also a considerable ornamental value (BONSTEDT 1931).

By virtue of the above considerations T. pannonicum has been introd troduced in the Agrobotanical Garden Cluj-Napoca in a series of experiments carried out with newly domesticated forage legumes (Astragalus cicer L., Coronilla varia L., Trifolium ambiguum M.B. etc.). Attention has been focused on infraspecific variability detected between and inside the population samples by means of biometry.

The main question was, why attempts for domestication failed, practically, on economically important scales. Domestication attempts influenced the infraspecific variability;then are there any microevolutionary trends due to past cultivation ?

This survey has been compiled with the intention of bridging some gaps still separating traditional botany, taxonomy, biometry, genetics, breeding and the study of evolution, in accordance with the principles outlined by CLAUSEN et al. 1940, DAVIS et HEYWOOD 1963, BRIGGS et WALTERS 1969, EHRENDRBER 1971, and now reflected in East European botany, too.

Polyploid evolution of true clovers toward gigantism - as opposed to allometric growth caused probably first and foremost by gene duplications (OHNO 1970, LAZÁNYI 1983, 1986) - was spectacular in the case of the species studied here. It is worth to note, that the section Stenostoma, where T. pannonicum belongs has been included by COOMBE (1968) in the section Trifolium, a solution which reflects that evolution acts here in the trunk line of the genus. Hovewer, the classification of JULEN (1959), and particularly that of BOBROV (1947) express the existance of a specific evolutionary trend, a reason why it was preferred here.

Polyploidy reached unexpectedly high levels in T. pannonicum: up to 2n = 22x = 180 ! Chromosome numbers of lower levels of 2n = 28, 49, 60, 65, 125, 126 and 130 have been also reported. Results from Romania published recently by N. TAYLOR (1987) indicate a chromosome number of 2n = cca 16x = 125.

Polyploid series have been reported for clovers outside the section and subsection, respectively as in the case of T. macrocephalum Pursch (2n = 32, 168), T. medium L. (2n = 48, 49, 63, 70, 72, 80, 126), T. burchellianum Ser. (2n = 48, 96). Tetraploid and hexaploid populations have been commonly reported in the case of some other species such as T. ambiguum MB., (2n = 16, 32, 48), T. attenuatum Green (2n = 16, 48), T. dubium Sibth. (2n = 16, 28, 32), T. fragiferum L. (2n = 16, 28), T. lupinaster L. (2n = 32, 40, 48), T. longipes Nutt. (2n = 16, 32, 48), T. repens L. (2n = 16, 32, 48), T. wormskioldii Lehm. (2n = 16, 32, 48) etc. (cf. CLEVELAND in TAYLOR 1985). The best studied case is perhaps that of the zig-zag clover (T. medium) where the genesis of euploid and aneuploid genomes have been followed in hybridation experiments, too (TAYLOR l.c.). Euploid values appeared here during the evolution perhaps partly by doubling of sets, partly by hybridation between plants (populations) on different ploydy levels. Aneuploid odd numbers of less than the multiple of the base can be explained by meioitic irregularities in sporogenesis. The situation is similar in the case of T. pannonicum, too (CLEVELAND l.c. and unpubl.).

If we suppose, that T.pannonicum emerged relatively recently -- perhaps during the inter-glacial and post-glacial periods -- not too far from, or even inside of the Transylvanian Alps (in a zone, where clover variablity is still high cf. 17,29,36,46) -- we may presume, that (micro)evolution is still active here. If it is supposed furtherely, that T. pannonicum can be integrated on GP-1 and/or GP-2 levels (22) in the common gene-pool of the subsection, it is quite clear that the polyploidization - hybridisation cycles may be still active in most sympatric situations (VIDA 1972, deWET 1980, MOHAY 1981). GAMS (in HEGI 1924) was probabily right in opinion that taxa described in the subsection as separate species belong -- in a biological sense -- to the same one. Such a high polyploid, as T. pannonicum is probabily an intermediate between a typical autopolyploid and a typical allopolyploid. The absorbtion of related forigen genomes is in any case enhanced, but also masked, by the polyploidy.

Polyploidisation may be also regarded, as an alternative possibility for the rapid increase of the DNA content per genome - a phenomenon accompanying frequently the domestication. (LAZÁNYI 1983, HAMMER 1985).

In such situations even the hybridisation is very difficult to test experimentally, due to the complicated dose effects and segregations so characteristic for high polyploids.

If the organism with the new genomic structure will compete more successfully for new habitats -- and chances are increased by hybridation and polyploidation , too - ecological isolation may occur and a new biological entity may emerge. Such an entity was identified and named, in 1767, by N. JACQUIN, with the name Trifolium pannonicum. It is worth to note, that habitat competition commonly tends to favour the emergence of perennials. Increased perennity vouches more generations of seeds and much more new seedlings for a successfully established new genotype fitted in a particular environment. In Transylvania the sympatric populations of T. ochroleucon Huds. (2n=2x=16) and T. pannonicum Jacq. (2n = cca 16x = 125,) clearly differ in habitat preference and cenological affinity (FUSS 1866 !, RESMERITA et al. 1973, KOVACS 1979 etc.).

Our experiments also revealed that T. ochroleucon is at best a short lived perennial (no accessions lived more than 2 years in our garden), whearas most T. pannonicum accessions are still vigurous 10 years after seeding.

With regard to comparative morphology between the two (and other) species and detailes for hystological and anatomical characters of the seedlings, leaves, petioles, stipels, epidermis, hairiness, vascular bundles etc. results published by C. TOMA (1966, 1969a,b, 1971, 1973) are of special interest.

Nodulation, widely recorded by many authors (ALLEN et ALLEN 1981), proved to be a highly variable character in our experiments.

Distribution and use

Among the clovers belonging to the subsection Ochroleuca the diploid T. ochroleucon Huds. has the widest geographical distribution, followed by T. caucasicum Tausch (included by COOMBE l.c. as a subspecies of the former taxon), and some other related taxa mostly also with uncertain taxonomic status: T. pallidum Jordan , T. trichocephalum MB. - this belonging to the same series as T. pannonicum - T. elongatum Willd., T. cassium Boiss., T. longidentatum Nabelek, T. davissi Hossein -- all distributed in more or less defined and restricted areas between the Caucasus mountains and Asia Minor. Few or no data have been found till now, regarding the polyploidy levels of this taxa (HENDRYCH 1968, 1970, CLEVELAND 1985 l.c.). T. pannonicum Jacq. s.s. is distributed on the western range of this areal-series, in E. Central Europe W. Ukraine, S.S.R. Moldavia westwards to E. Austria and N. Italy, respectively between S. Poland and N. Albania.

Whether hybridation acted or not , between the members of the subsection on interspecific or intraspecific level, is perhaps in most cases a matter of pure convention. This opinion is supported by solutions adopted by GAMS, COOMBE and others, too. Intermediate forms described by different botanists (HOLUBY 1888!, GIBELLI et BELLI 1888!, TRAPL 1923, GAMS in HEGI 1924 pg. 1334, DOMIN et PODPERA 1928 ap. HENDRYCH 1968, SĂVULESCU et RAYSS 1934, BORZA 1949, NYÁRÁDY 1959) seems to deserve more attention, as received earlier.

Except of a refference of FLEISCHER in DOMIN mnscr. (ap. HENDRYCH l.c.) pg. 160), no references regarding the cultivation or breeding of Hungarian clover have been found till now for the countries inside the natural distribution area of the species, as for Bulgaria (KOŽUHAROV 1978), Czechoslovakia (HENDRYCH l.c., et Bibliographia Botanica Czechoslovaka), Hungary (SOÓ 1962-1982, PRISZTER 1985, BÁNYAI 1983), Romania (NYÁRÁDY 1959, RESMERIȚA et al. 1973) etc. But on the other hand -- in accordance with the general trends observed in plant domestication -- cultivation has been recorded frequently outside the area of the natural distribution of the species: first from Germany (DIERBACH 1839 !, GAMS in HEGI 1924, BONSTEDT 1931, MANSFELD 1959, PETERSEN 1966, OBERDORFER 1970, SCHULTZE-MOTEL et al. 1986, in press.), later from Switzerland (STEBLER 1913), Austria (JANCHEN 1960), Czechoslovakia (MUNZAR ap. HENDRYCH l.c.), France (FOURNIER 1961 ap. l.c.). Breeding started first perhaps in 1888 at the Seed Testing Station at Zürich (Switzerland, STEBLER l.c.) in order to substitute alfalfa with a perennial gigant clover adapted to mountain climates. These experiments proved that T. pannonicum needs for good yields deep, nutrient rich soils , and yearly rainfall of about 800 - 1000 mm, irrespective of the drought resistance, assumed for the species. Founder populations strongly influenced results and desired population characters were difficult to fix, presumabely. Stands had a slower regrowth after harvest, as comared with white or red clover. After the first year - and right technology - two yields can be harvested yearly and yields remain constant over a long period of time - in our case for up to 6-10 years. So this is perhaps the most long lived perennial clover (except white clover) ever used for hay. It has a good palatability for cattle, it is often consumed by the wild, irrespective of the hairiness ( ). Once estabilished persists for decades, even subspontaneously.

The flowers of the studied plants have been almost completely self-sterile and needed open pollination on population level. Pollination has been performed mostly by bumblebees. Despite of the high nectar production (2,7 mg nectar per flower, as compared with 0,5 mg for T. repens and about 1 mg for T. pratense) , the plant has little if any apicultural value, as the corolla tube is too long for the honey-bee (NYARADY 1958).

Seed weight varied between 2500-4500 mg per 100 seeds for the different accessions of T. pannonicum as compared with 1400-2000 mg found for T. ochroleucon. (Fig. 1). Seed production is generally low (about 120 kg/ha) due perhaps to the lack of pollinators and high percentage of sterile flowers. Hard seed content was surprisingly low (about 80 % on average), but scarification was very effective for quick seedling development. Seed quantity recommanded for seeding varied between 20-30 kg/ha.

Seeds are consumed by different seminifagous insects: Bruchus sericatus, Bruchofagus gibbosus, Apion aestivum etc. (PERJU 1985).

Our results regarding the nutritive value of the hay, palatability, compatibility with tall fescue in legume:grass mixtures (unpubl.), lower susceptibility to radioactive contamination due perhaps to the hydrophobe properties of the leaf surface (SZABÓ et TAMÁS 1986), as well as other reasons make the species still promising for attempts of further domestication and breeding.

In vitro techniques my be useful tools in the future, for the augmentation of selected populations, for the multiplication of desired genotypes in large, uniform stands.

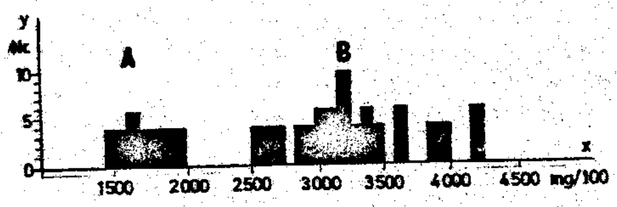


Fig. 1. Seed weight measured by the accessions collected from nature and/or obtained from different botanical gardens and preserved in our collection. A = T. ochroleucon Huds., B = T. pannonicum Jacq. X = mg per 100 seed, Y = number of samples.

T. pannonicum was included in the category of cultivated plants by ZEVEN et ZHUKOVSKY(1975), SANCHEZ-MONGE (1980), but not by JULEN (1959), DUKE (1981), TOWNSEND(1985).

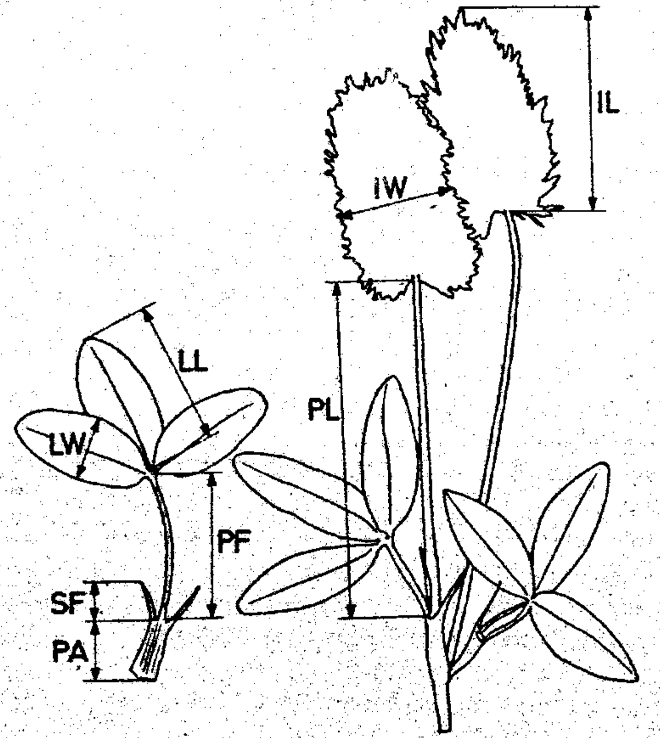


Fig. 2. Biometrical measurements performed on Trifolium pannonicum Jacq.:

IL = inflorescence length
IW = inflorescence width
LL = middle leaflet length
LW = middle leaflet width
PA = petiol, length of the adnate part
PF = petiol, length of the free part
PL = peduncul length
SF = stipel, length of the free part

Notes: 1. LW is indicated here for technical reasons on a lateral leaflet
2. All measurements in millimeters /mm/

Results

1. Herbarium collections. Taxonomy

Five major herbarium collections have been studied for this paper: HUC - the Herbarium of the University Cluj-Napoca, CLA - the Herbarium of the Agronomy Institute Cluj-Napoca, BUA - the Herbarium of the Agronomy Institute București ,HUI - the Herbarium of the University Iași and HIA - the Herbarium of the Agronomy Institute Iași. 176 *T. pannonicum* specimens preserved in these herbaria have been measured: 180 specimens originated from Transylvania, 26 from Moldavia and 20 from Austria (AU), Czechoslovakia (CS), Hungary (HU), and Poland (PL), mostly from the northern limits of the distribution area of the species. The Transylvanian collection was big enough to be divided randomly in three sub-samples of 50, 50 and 30 specimens each, in order to compare the differences found by the calculated statistical indicators not only between, but also inside the territories (Table 2,3).

Table 2

Comparation of mean values calculated for the measured characters in collections grouped according to the regions. For character codes see Figure 2.

Nr.	Region	PA	PF	LL	LW	SF	LS	PL	IL
1.	MOLDAVIA	21,15	48,30	46,69	12,50	23,84	12,26	64,65	49,11
2.	TRANSYLVANIA	14,69	41,66	48,75	12,32	21,11	12,61	44,58	43,44
3.	AU,CS,HU,PL	12,76	34,23	48,61	12,33	22,05	12,57	63,19	41,76
	x	16,20	41,40	48,02	12,38	22,33	12,48	57,47	44,77
	s	4,40	7,04	1,15	0,10	1,39	0,19	11,19	3,85
	s%	27,16	16,99	2,40	0,80	6,21	1,50	19,47	8,60

Values in the Table 2 indicate the existance of some differences between the means registred for different geographical areas. The largest differences have been registred in the length of the adnate part of the stipel and in the length of the inflorescences. Differences have been small in the length (LL) and width (LW) of the

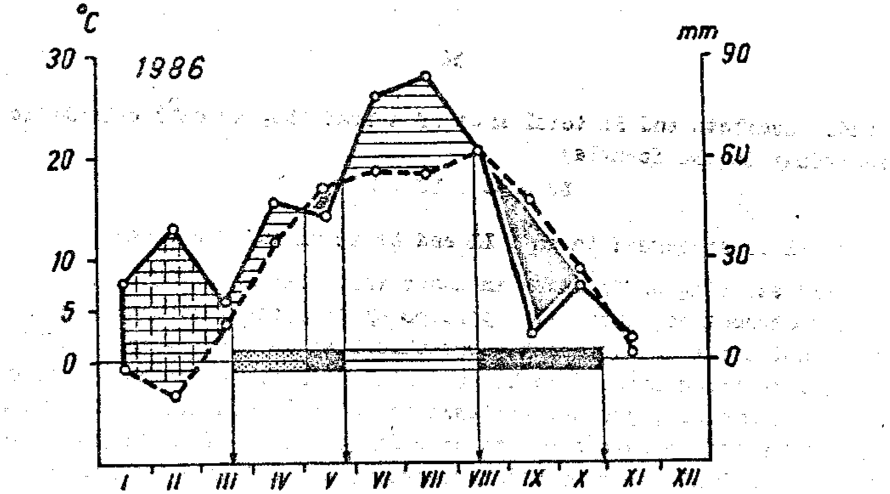


Fig. 4. Diagram of the climatic data for the year 1986

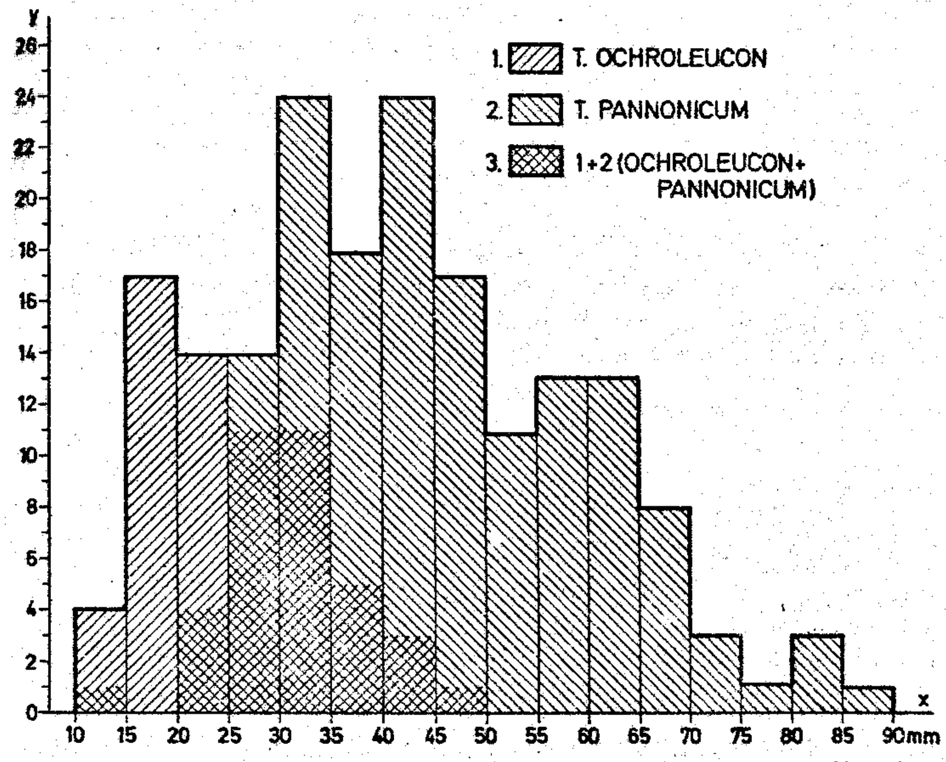


Fig. 4. Hystogram of the measured herbarial specimens according to median leaflet length /x/; y = number of specimens in the category.

middle leaflets and in total area of a leaf (LS, in cm^2) calculated according to the formula:

$$LS = LL . LW . K$$

where LS is expressed in cm^2, LL and LW in mm and K = 0,021

The existing differences indicate the existence of specific quantitative characters, controlled perhaps by polygenes, accumulated in different doses in different geographical areas (cf. LAZANYI 1987). Such differences are difficult to define by means of classical taxo-
Such differences are difficult to define by means of classical taxonomy, but may have great importance in (micro)evolution. Such quantitative characters have also economic importance, influencing not only production, but also some other traits, such as longevity and resistances. So polyploid series may form useful experimental tools for the understanding of the plant longevity - inconceivably neglected in plant biological studies.

Comparing the means and the coefficients of variation (x and s%) obtained in the case of Transylvanian sub-samples, with those found for Moldavia, Northern Carpathians and for the whole territory (Table 4) it is quite clear, that s and s% values are consistently higher for the Transylvanian sub-samples. This is caused perhaps not only by the differences in the number of measured specimens.

Table 3

Comparation of the standard deviation (s) and the coefficient of variation (s%) calculated for the Transylvanian sub-samples and for the three sampled regions, respectively. Symbols as in Table 3, Fig. 2.

Nr.	Region	PA s	PA s%	PF s	PF s%	SF s	SF s%	PL s	PL s%
1.	TRANSYLVANIA	3,17	2,60	5,66	13,60	1,74	8,24	0,71	1,60
2.	For the whole area studied	4,40	27,16	7,04	16,99	1,39	6,21	11,19	19,47

As differences have been highest for the Moldavian plants, which are distinct from the Transylvanian populations for some other traits, too, a new variety - var. moldavicum var. nova - have been described here (see Table 4, Addenda).

The study of evolutive connections between the Moldavian populations still belonging to *T. pannonicum* Jacq. and that of other taxa, described as different species from more Eastern parts of Europe and from Asia Minor, respectively, needs further research. By all means the varieties described by SAVULESCU et RAYSS (1934) under the names var. *aureum* and var. *sericeum* perhaps belong to intermediate topodemes and may be regarded as "missing links" for *T. pannonicum* s.l.

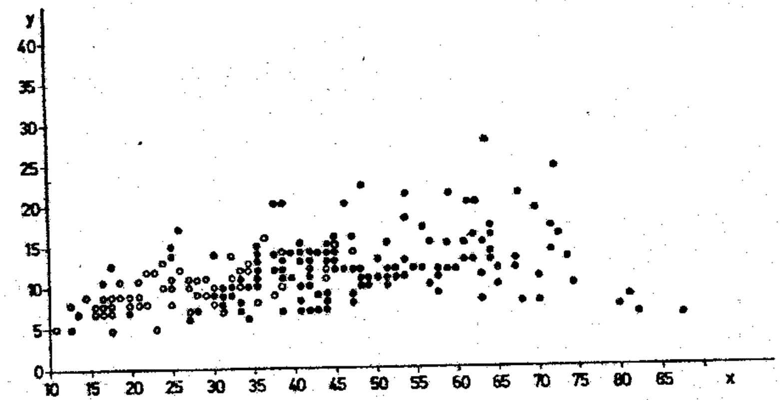


Fig. 5. The dispersion diagramm of the studied herbarium specimens according to the characters "middle leaflet width" (LW on Y) and "middle leaflet length" (LL on X) cf. Fig. 2 too.

2. Variability in spontaneous population (in nature)

The intrapopulational variability of the characters studied on herbarium specimens has been checked in a spontaneous population sample collected from Stana, distr. Cluj, alt. s.m. cca 580 m, map 22, pixel coordinates 66.59 on DI scale cf. SZABÓ (1986). The habitat which preserved the population is an ecotone between a grassland (*Festuceto rubrae - Agrostidetum tenuis*) and a mixed oak forest (*Quercetum petraeae-cerris*). There are plenty of individuals, *T. pannonicum* forming here a well identified facies, similarly to those described by M.I. TOMA of Suceava district.

Measurements have been made in three different conditions, named here "sampling situations": 1. on randomly collected plants (RAND), 2. on best developed plants and plant organs (DEVL), and 3. on individuals digged up randomly and transplanted in the agrobotanical garden, measured six month after the transplantation (TRAN). 36 measurements have been made for each voucher specimen preserved in CLA.

Table 4

Trifolium pannonicum Jacq. - a survey of described taxa

Genus	TRIFOLIUM L.
Subgenus	TRIFOLIASTRUM Ser. in DC.
Sectio	STENOSTOMA Gib. et Bel.
Subsectio	OCHROLEUCA Bobrov
Series	TRICHOCEPHALUM Bobrov
Species	TRIFOLIUM PANNONICUM Jacq.

acutifolium Schur 1866 - SCHUR 1866 pro (var.), NYÁRÁDY 1957 pro f., syn. f. carpaticum Kárp., SOO 1968 pro var. (ssp. ?) carpaticum Kárp.

alopecuroides Pers. - PERSON 1807 non KITAIBEL , pro sp., MANSFELD 1959, syn. pro T. pannonicum Jacq. (=T.p.), SCHULTZE-MOTEL et al. 1986 idem.

angustifolium Schur - SCHUR 1866 pro (var.), NYÁRÁDY 1957 pro f., HENDRYCH 1968 idem.

angustifolium Vandas - VANDAS pro ?, BECK-MANAGETTA 1927 pro (var.), HEDRYCH 1968 idem.

armenium Baumg. - BAUMGARTEN 1816 pro sp., syn. T.p. auct. div.

armenium auct. non Willd. - syn. T.p., cf. SIMONKAI 1888, HEGI 1924, HENDRYCH l.c.

aureum Săv. et Rayss - SĂVULESCU et RAYSS 1934 pro var., BORZA 1949 pro f.,HENDRYCH l.c. pro var.

canescens Willd. - WILLDENOV pro sp., GAMS in HEGI 1924 pro ssp.,T.p.

carpaticum Kárp. - KÁRPÁTI 1935 pro f. (n.n.), NYÁRÁDY 1957 syn. pro f. acutifolium Schur, SOO 1964, 1968 pro ssp., HENDRYCH l.c.,f.

cassium auct. non Bois. - SĂVULESCU et RAYSS 1934(notomorpha), hybrid T. ochroleucon x T. pannonicum, BORZA 1949 idem, NYÁRÁDY 1957 idem , GIBELLI et BELLI 1888 hybrid cf., HENDRYCH 1968 syn. pro T. ochroleucon Huds. f. majus Holuby (?).

cassium Boiss. - BOISSIER 1849 pro sp., GIBELLI et BELLI 1888,pro 1889 hybrid (nm. = notomorpha) T. ochroleucon x T. pannonicum , SĂVULESCU et RAYSS 1934 idem, NYÁRÁDY 1957 idem, HEDRYCH l.c. pro sp. !

davidovii Kož. - KOŽUHAROV 1976 var. pro ssp. pannonicum

elongatum Willd. - WILLDENOW pro sp., SIMONKAI 1888 syn. pro T.p., GAMS in HEGI 1924 ssp. pro T.p.

foliosum Sz.T.A., h.l. - convar. nova, cf. Addenda.

hohenackeri Jaub.et Spach - GAMS in HEGI 1924 pro sp.(ssp.) pro T.p.

lancifolium Beck. - BECK-MANAGETTA 1927 pro f., HENDRYCH l.c. idem.

latifolium Schur - SCHUR 1866 pro (var.), NYÁRÁDY 1957 pro f., HENDRYCH l.c. idem, KOŽUHAROV 1976 f. pro var. pannonicum.

michaelis-borsii Kárp. - KÁRPÁTI 1940 pro f., idem auct. div.

major Holub. cf. T. ochroleucon

minus Beck. - BECK-MANGANETTA 1927 pro f., HEDRYCH 1968 idem.

moldavicum Sz.T.A.,h.l. - var. nova, hoc loco, cf. diagnosis in Addenda.

ochroleucum Gen. - GENERS 1801 non HUDSON , syn. pro T.p.

ochroleucon Huds. f. pannonicum Zanov. - V. ZANOVSCHI in herb. HIA, ? T. x cassium Boiss. sec. NYÁRÁDY syn. T. ochroleucon f. major Holub, nm ?

ochroleucon Huds. f. major Maloch - MALOCH 1932 syn.pro T.p. ap.HENDRYCH

olympicum Hornem. - HORNEMANN pro sp. vel. ssp. pro T.p. cf. HEGI 1924.

pannonicum Jacq. - N. JACQUIN 1767 in Observ. Bot. 2:21; L.,Mantissa Pl., ed. 2, 276 (1771), non ENDLICHER nec LUMNITZER, VILLARS, FEDCHENKO etc. Iconotypus: JACQUIN 1767, Obs. Bot. 2, tab. 42.

pannonicum L. - syn. pro T. pannonicum Jacqu cf. SIMONKAI 1888, HEGI 1924, SOO 1968 cor. 1970.

pannonicum Vill. non Jacq. - sp. in HEGI 1924 vol. IV/3 pg. 1334, syn. T. pratense L. var. frigidum Gaudin non Schur f. lutescens Rouy ; an vero?, notomorpha ?

rubro(i)calycinum Podp. - PODPERA 1902 pro var., HENDRYCH l.c. idem, KOŽUHAROV l.c. var. pro ssp. pannonicum.

sericeum Săv. et Rayss - SĂVULESCU et RAYSS l.c. pro var., HENDRYCH l.c. idem.

sulphureum Koch - in HEGI 1924 pro sp., vel ssp. pro T.p. (?)

typicum Beck. - BECK-MANAGETTA 1927 pro f. , syn. ssp., var. et f. pannonicum cf. KOŽUHAROV l.c.

traplii (Trapl)Podp. et Dom. - TRAPL 1923 pro T.p., DOMIN et PODPERA 1928 pro(nm.) T. alpestre x T. pannonicum , HENDRYCH l.c. lusus pro T.p., an vero ?

A D D E N D A - Diagnoses taxonum novarum

Trifolium pannonicum Jacq. var. moldavicum var. nova - Inflorescentia magna vel 80-100 mm longa, medio caule petiolem equans, caulibus, stipulilis et petiolis dense aureo-villosis /vide var. aureum Săv. et Rayss, var. sericeum Săv. et Rayss/. Distributio: in Moldavia. Holotypus: in Herb.Inst.Agr. Iaşi. Leg. in Moldova,Andreiaşu de Jos,24.VI.1956,Răvăruţ.

T. pannonicum Jacq. convar. foliosum convar. nova. Plantae robustae erectae, dense foliosae, foliolis cca 2,5-3 longioribus quam latis, saturate-viridibus. Germinatio seminorum 75-95%. Formae cultae. Holotypus in Herb. Inst. Agr. (CLA) nr. 28.200 . Leg. Szabó T.A.

Results regarding the main statistical indicators (minimum, maximum, mean, standard deviation, coefficient of variation) are presented in Table 5. The highest standard deviation (s) have been registered for peduncul length, the lowest for leaflet width, irrespective of sampling situation (RAND, DEVL or TRAN). Sampling situation influenced the length of the free part of the petiol and the inflorescence size, preponderently. The coefficient of variation (s%) was the highest by the transplanted plants. Sampling (RAND, DEVL) influenced statistical indicators in all cases except the free part of the stipel.

Table 5.

Main statistical indicators (m,M,x,s,s%) which reflect the intrapopulational variability of measured characters (see Fig. 2) in a spontaneous population of T. pannonicum Jacq. (Stana, jud. Cluj)

Indicators SAMPLING	Characters PA	DF	LL	LW	SP	LS	PL	IL	IW
Minimum (m)									
RAND	15,3	13,3	43,0	9,3	13,3	9,3	36,0	35,0	27,0
DEVL	18,0	18,0	53,0	8,0	15,0	10,1	30,0	38,0	30,0
TRAN	8,3	15,3	23,0	8,5	5,3	5,5	16,0	25,0	26,5
Maximum (M)									
RAND	31,3	37,7	68,3	15,0	34,3	20,5	100,0	67,5	44,7
DEVL	45,0	58,0	62,0	17,0	38,0	24,2	100,0	57,0	54,0
TRAN	36,7	84,0	39,7	17,0	14,3	14,2	45,0	30,5	30,0
Mean (x)									
RAND	20,3	25,1	54,5	11,2	22,0	14,4	60,8	47,1	37,3
DEVL	27,3	39,8	60,8	13,2	24,3	16,5	69,1	52,1	35,9
TRAN	14,8	34,1	30,1	12,9	9,8	8,2	30,0	28,5	28,8
Stand.dev. (s)									
RAND	4,60	7,41	6,93	2,26	6,28	3,99	18,25	8,98	5,14
DEVL	5,65	10,92	5,86	2,48	6,84	4,17	18,89	6,93	7,30
TRAN	8,11	22,08	5,52	2,98	2,93	2,86	14,53	3,04	2,02
Coef. var. (s%)									
RAND	22,67	29,55	12,71	20,25	28,61	27,74	30,01	19,07	13,76
DEVL	28,02	27,41	9,64	18,78	28,14	24,63	27,33	13,30	20,33
TRAN	54,79	64,51	18,35	23,11	28,90	34,79	48,42	10,67	7,01

3. Selected plants

3.1. The starting collection (Table 1) has been biometrized in the second year after seeding, in 1978, for the conomically important plant characters as plant hight (PH), stem number per plant (SN), stem dry matter per plant (SD), leaf dry matter per plant (LD), stem:leaf ratio (S:L), inflorescence number per plant (IN), flower number per inflorescence (FN). Results are presented in Table 6. The leaf cha-

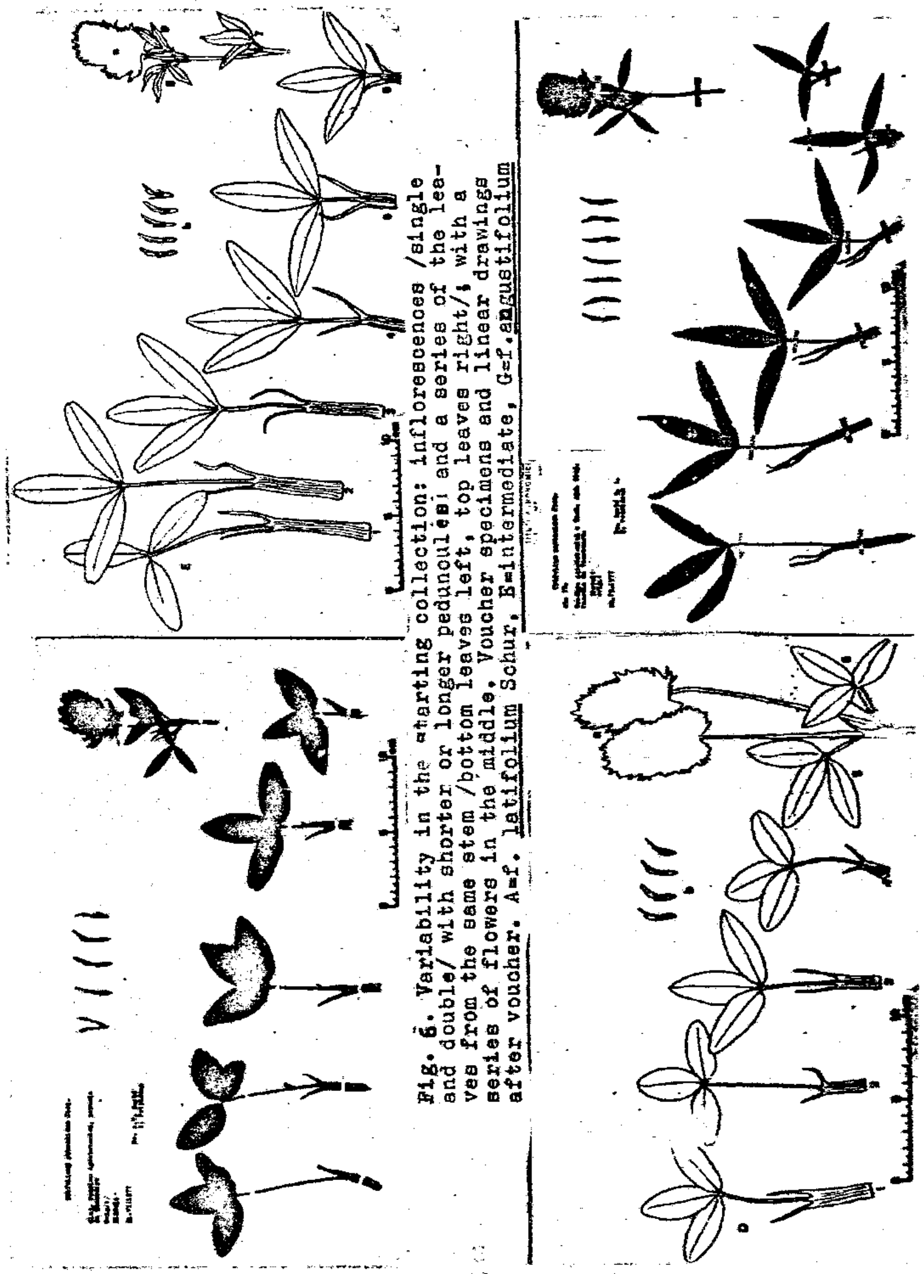


Fig. 6. Variability in the starting collection: inflorescences /single and double/ with shorter or longer peduncules; and a series of the leaves from the same stem /bottom leaves left, top leaves right/; with a series of flowers in the middle. Voucher specimens and linear drawings after voucher. A=f. latifolium Schur, B=intermediate, G=f. angustifolium

racters have been measured as in herbarium samples (cf. Table 2,3). Results for leaf characters are tabulated in Table 7.

Table 6

The variability of economically important plant characters measured in 1978 in the starting collection of Trifolium pannonicum Jacq., cultivated in the Agrobotanical Garden

Indicators	Characters						
	PH	SN	SD	AD	S:L	IN	FN
Minimum (m)	65,0	14,0	25,0	17,0	1,03	16,0	70,0
Maximum (M)	96,0	60,0	67,0	45,0	1,61	48,0	97,0
Mean (x)	80,3	30,6	45,1	32,9	1,37	33,7	83,4
Stand. dev.(s)	8,57	11,87	12,29	9,03	0,43	10,96	8,47
Coef. var. (s%)	10,67	38,79	27,25	27,44	31,38	32,52	10,15

Note: for character symbols see the text

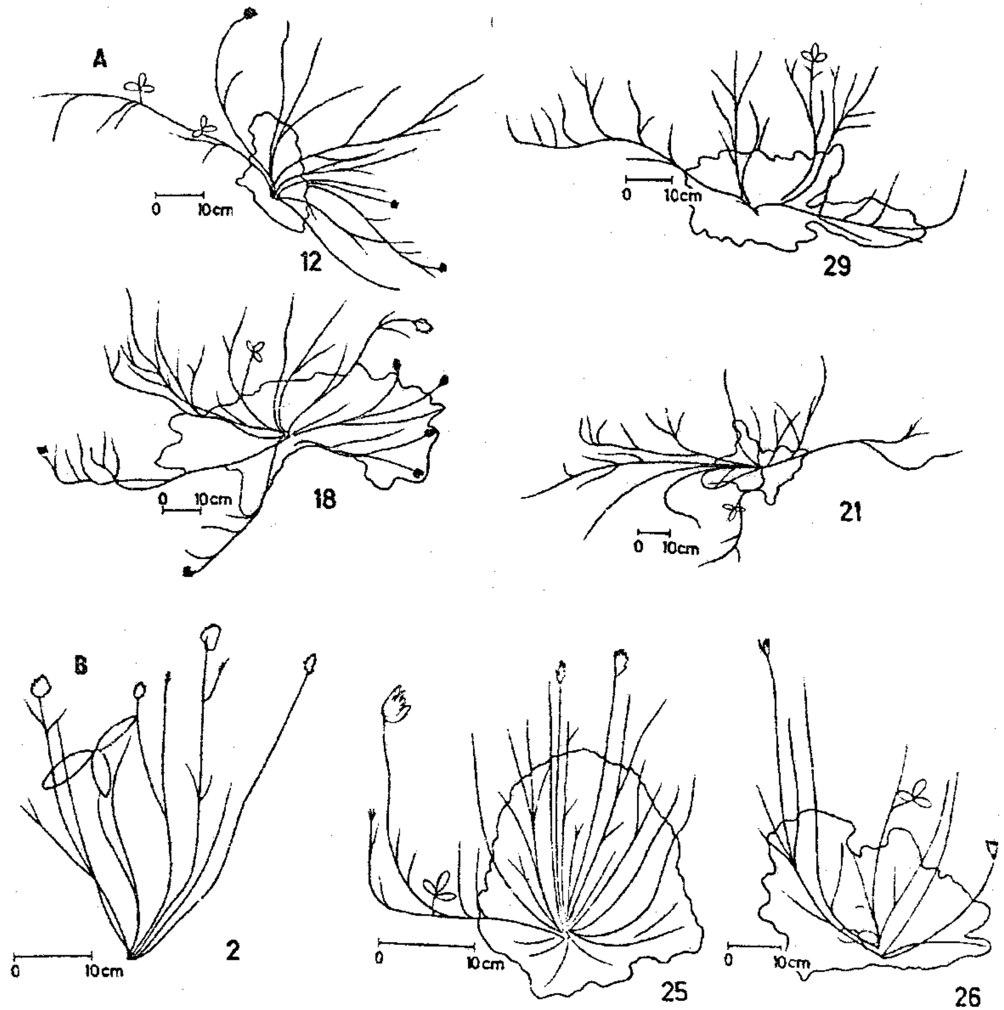
For plant characters the largest coefficient of variation has been found for stem number per plant (38,79%) , correlated of course positively with inflorescence number per plant. The starting collection was most uniform for plant hight (s% = 10,67).

Leaf characters (Table 7 , Fig.2, 5) have been variable for the adanate part of the petiol with the stipel (PA, 27,9%), for the free part of the petiol (PF, 26,6%), and surprisingly uniform for leaf length (LL, 12,9%), but not for leaf surface (LS, 20,53%).

Table 7

The variability of the leaf characters measured in 1978 in the starting collection of T. pannonicum Jacq. cultivated in the Agrobotanical Garden (symbols as in Fig. 2)

Indicators	Leaf characters					
	PA	PF	LL	LW	SF	LS
Minimum (m)	21,0	29,0	54,0	14,0	19,0	17,6
Maximum (M)	50,0	64,0	81,0	26,0	46,0	32,2
Mean (x)	34,1	44,5	65,5	19,2	31,1	24,1
Stand. dev. (s)	9,53	11,23	8,50	3,92	8,29	4,95
Coef. var. (s%)	27,95	25,23	12,97	20,42	26,65	20,53



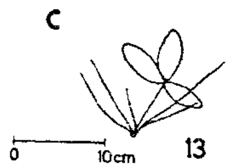


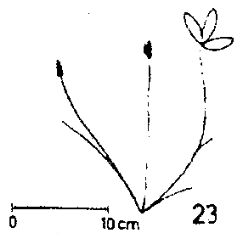
Fig. 7. The variability of growth form in a selected population of open pollinated plants in the se - cond year:

A: prostrate types /12,18,21,29/

B: erect types /2, 25, 26/

C: dwarf types /13, 23/

Note: Main stems, the outline of a leaf, that of the [illegible] foliage, and inflorescence with flowers /white/ or with seeds /black/ are indicated. Date: [illegible]



3.2. Elite plants have been selected for persistence and resitance by natural selection acting upon the starting collection in the period between 1976 - 1984. During this period firstly disappeared all diploid T. ochroleucon accessions and aobout 50 % of T. pannonicum accessions, but this mostly after the fifth vegetation year.

At the end of the 8 year selection period well developed plants,-- more resistant to the heavy infection with fungal diseases (agents not identified) characteristic for the experimental plot -- have been selected as "elites" (E). These elite plants formed an open pollinated population and elite seeds have been harvested in autumn 1984. This elite population showed a slightly larger coefficient of variability for most of the traits, as compared with the starting one, due perhaps partly to the smaller sample size, preserved extremes and better developped (older) individual plants. (Table 8).

Table 8

The variability of some plant characters (plant hight = PLH and number of inflorescences per plant = IPL) measured and calculated in 1986 for the elite plants derived from the original, starting collection cultivated in the Agrobotanical Garden

Nr.	Symbol for elite plant	PLH	IPL	Flowering time	Origin of the elite
1.	E-04-76/86	80,0	20,0	late	Brüxelles
2.	E-06-76/86	72,0	102,0	early	Aachen
3.	E-08-76/86	92,0	51,0	intermediate	Oldenburg
4.	E-11-76/86	100,0	105, 0	late	Cluj-Napoca
5.	E-12-76/86	95,0	95,0	early	Warszawa
6.	E-13-76/86	95,0	97,0	intermediate	Berlin
7.	E-14-76/86	75,0	73,0	"	Bucureşti
8.	E-18-76/86	85,0	88,0	late	Frankfurt a.M.
	Mean (x)	86,75	78,89		
	Stand.dev.(s)	10,30	29,68		
	Coef. var. (s%)	11,88	37,62		

3.3. Progenies obtained from elite seed germinated on paper, planted first in plastic pots and later, in a three leaf stage,in the open in the Agrobotanical Garden (May 1985), dug out in May 1986 and underground parts washed carefully have been also measured for stem and root characters. The following traits have been measured: main root length = RL in mm; secondary root number = RN (roots with a diameter of 5 mm or more have been considered); collar or "crown" diameter = CD in mm; root nodule number in a 5 cm long portion charcteristic for the plant;= NN; plant hight measured as stem length = PH in mm; stem number per plant = SN

The size and colour of the root nodules have been also registered but not introduced in the Table 9. The coefficient of variability was striking in the case of secondary root number (s% = 87,7 %), correlated positively with nodule number per root (s% = 73,7%). This draws attention on an important selection criteria, too.

Table 9

Root and stem variability in 29 one-year-old elite progenies of T. pannonicum Jacq. before the transplantation in the experimental field Sapca Verde (8.V.1986). For characters symbols see the text part.

Indicators	RL	RN	CD	NN	PH	SN
Minimum (m)	45,0	0,0	4,0	2,0	70,0	2,0
Maximum (M)	300,0	18,0	20,0	30,0	350,0	18,0
Mean (x)	201,2	5,2	9,5	8,6	189,1	6,7
Stand.dev.(s)	56,15	4,56	3,78	6,34	75,45	3,95
Coef. var.(s%)	27,90	80,69	39,78	73,72	39,89	58,95

The results of the further biometrical measurements have been negatively influenced by a series of factors: heavy sommer droughts (see Fig. 3), oped field habitat, mycoplasma infection transfered by insects from a neighbouring white clover field (Fig.), and even by the transplantation itself. This explains, perhaps, why popolation means remained well behind the means registered in the starting population of similar age, in 1978. Besides, elites have been selected only for persistence and resistance to adverse natural conditions, but not for plant habit or leaf size. The mean plant height measured not as stem length, but as above ground height, was 29,49 cm (m= 5 cm, M = 55 cm, s = 12,95, s% = 43,98) indicating a tendency for prostrate habit (see Fig.8-16-25). The mean number of stems per plant was 11,21 (m = 4, M = 25, s = 5,53, s% = 47,22). Flowering was higly variable about 10 % of plants still flowering in late September.

In Table 10 leaf characters are compared for selected progenies as measured on Sept.20,1986, in a similar manner, as in Table 5, but instead of transplanted plants, plants infected with mycoplasma (MYCO) have been studied here. Values have been compared with that registered for the starting population (START) in 1978.

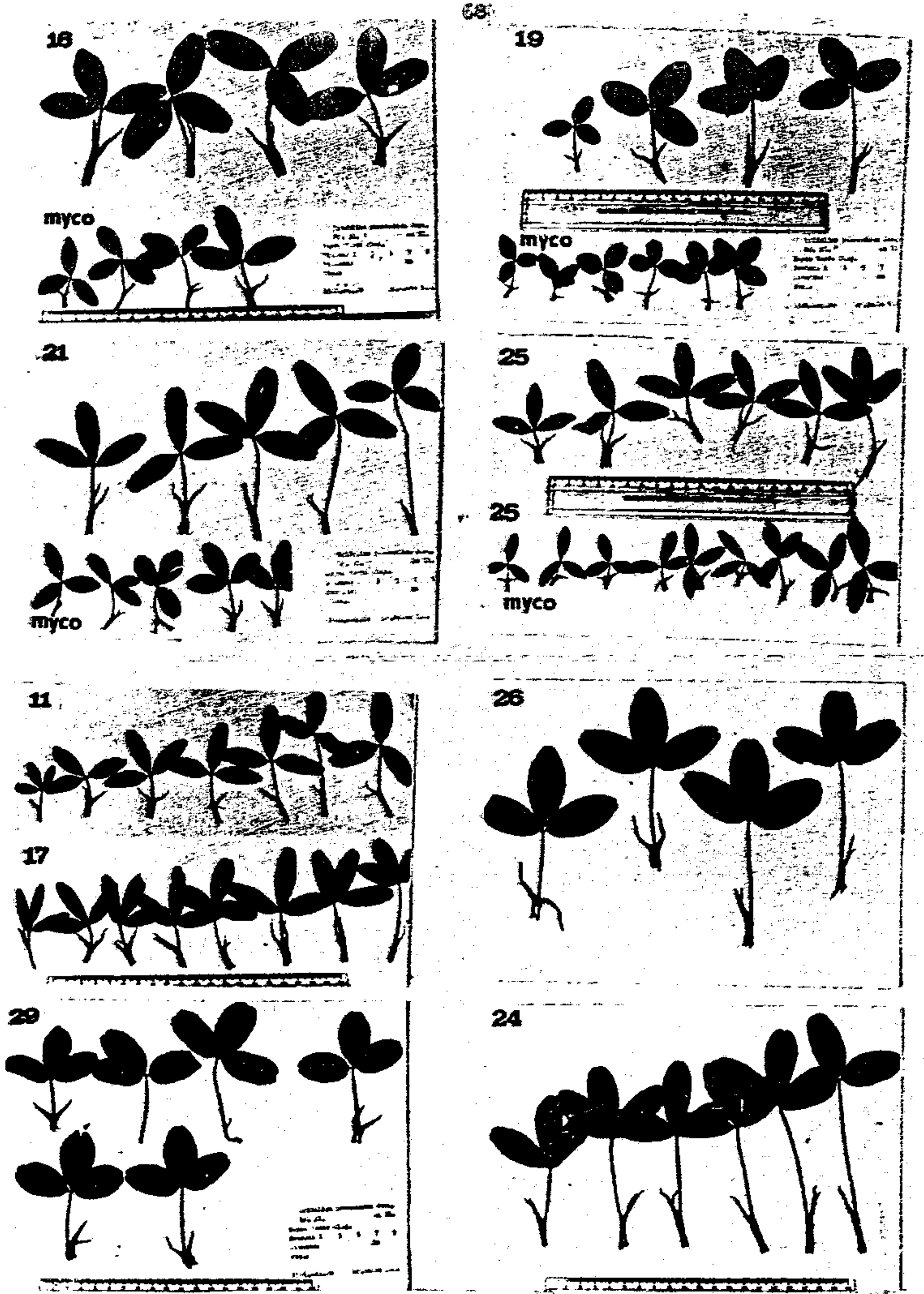


Fig. 8. The variability of the leaf /a series from the main stem/ in the same population as that represented in Fig. 7. /plants nr. 21, 25 and 26 are also the same/. MYC = leaves from mycoplasma infected stems of the same plant.

Table 10

Comparation of minimum (m), maximum (M), mean (x), standard deviation (s) and coefficient of variation (s%) registered for the starting population (START) and selected progenies sampled for best developed organs (DEVL), sampled randomly (RAND) and for plants infected by mycoplasma (MYCO). For the character symbols see Figure 2.

Indicators SAMPLING	Characters					
	PA	PF	LL	LW	SL	SF
Minimum (m)						
START	21,0	29,0	54,0	14,0	19,0	17,6
DEVL	15,0	22,0	41,0	14,0	11,0	10,6
RAND	11,6	10,7	29,0	10,7	11,3	7,7
MYCO	7,0	8,0	21,0	9,8	7,7	4,7
Maximum (M)						
START	5o,0	64,0	81,0	26,0	46,0	32,0
DEVL	33,0	74,0	80,0	27,0	50,0	32,5
RAND	27,9	62,2	64,7	26,5	36,7	29,9
MYCO	16,2	29,4	47,6	20,0	21,0	20,0
Mean (x)						
START	31,1	44,5	65,5	19,2	31,1	24,1
DEVL	20,5	40,6	44,8	16,4	19,5	18,7
RAND	17,3	30,9	40,7	16,8	17,8	14,8
MYCO	10,8	17,0	30,8	12,6	12,3	8,8
Standard dev. (s)						
START	9,53	11,23	8,50	3,92	8,29	4,95
DEVL	5,41	16,62	9,85	4,73	4,40	7,36
RAND	4,27	14,16	9,51	9,70	5,80	6,45
MYCO	2,56	5,72	8,87	2,91	4,98	4,49
Coef. of variation (s%)						
START	27,95	25,23	12,97	24,32	26,65	20,53
DEVL	26,39	40,93	21,98	25,76	22,56	39,02
RAND	24,65	45,89	23,39	57,64	32,69	43,66
MYCO	23,76	33,64	28,77	23,09	40,48	52,49

Standard deviations and coefficients of variation were generaly lower for starting population as for selected ones, except MYCO plants. For MYCO plants especially petiol length and leaf surface were reduced.

The coefficient of variation were the largest in the case of petiol length, leaflet width (RAND) and leaf surface (MYCO), respectively. In the last case variability had been influenced by the fact, that not all leaves showed the same degree of demage on an infected and sampled stem.

The growth form of the elites varried between very small and very large erect plants without or with considerable leaf biomass production (Fig. 7,nr. 12,21) semiprostrate (Fig. 7,nr.2) and prostrate or even erect types (Fig. 7,nr.12,15).

The economic aspects of T. pannonicum cultivation needs further research. In our trials - pure culture and mixture with Festuca arundinacea (F.A. : T.P. = 10 : 1) - the brutto protein content of the hay was very similar to that of white clover (Table 11), but with a considerably higher crude cellulose content. In mixed sward the clover proportion used in our experiments did not improve substantially the quality of the hay.

Table 11

The protein and cellulose content of different Trifolium pannonicum accessions in pure culture and in mixed sward (see text) in hay harvested from the experimental field Sapca Verde

Sample	Crude protein content (% DM)	Crude cellulose content (% DM)	Analyzed part
TRIPAN-1	18,24	24,94	whole plant
TRIPAN-2	17,65	24,32	" "
TRIPAN-3	18,37	25,73	" "
TRIPAN-4	17,18	25,10	" "
$\bar{X}$	17,86	25,02	" "
TRIPAN-5	15,65	25,88	stem
	20,15	22,28	leaf
Mixed sward	10,25	25,26	F.arundinacea: T.pannonicum (10:1)
Trifolium repens cv.TATAN (martor)	18,90	18,33	brutto harvest (leaf!)

Our results are in general agreement with that of STEBLER (1895), PETERSEN (1967), RESMERIŢA et KAIN 1969 (ap. RESMERIŢA et al. l.c.), regarding the nutritive value of T. pannonicum hay. It seems, that the species is worth of interest as a companion legume for perennial, polyploid grasses such as tall fescue.

Conclusions

1. According to the available data polyploidization in Trifolium reached the highest level in T. pannonicum Jacq./Sect. Stenostoma, subsect. Ochroleuca/. The critical review of early taxonomical results in the light of the polyploid evolution process seems to be necessary.

2. T. pannonicum, collected from natural, spontaneous populations and preserved in different herbaria reflects some microevolutive differentiation between the eastern and western populations. These differences are worthy of taxonomical treatment.

3. The species is preadapted for domestication. It has been cultivated first in the early 19th century outside of its natural distribution area /Germany/. Mass selection and breeding for agricultural purposes started in 1888 /Switzerland/.

4. Economically relevant cultivation failed perhaps even due to the breeding difficulties caused by high levels of polyploidy and the inadequate genetic resources available for breeding.

5. Domestication influenced infraspecific variability. Latifolium type populations with agronomically more valuable characters, better germination, higher leaf:stem ratio, better regrowth after harvest and increased perennity emerged and the outlines of a new, cultivated convariety /convar. foliosum/ appeared.

6. T. pannonicum may be of interest as a polyploid companion legume in mixed swards of perennial polyploid grasses such as Festuca arundinacea. The high coefficient of variation found in the case of some economically important traits may be of interest in further breeding.

7. The continuous increment of genome size by polyploidization -- as opposed to intracromosomal increment of genome size due to gene duplications -- makes this species of interest for theoretical studies, too.

Rezumat

SZABO T.A.,1987, Microevoluţie în genul Trifolium sect. Stenostoma I. Biometria speciei T. pannonicum Jacq. spontan şi cultivat.

. În genul Trifolium specia T. pannonicum a ajuns la nivele cele mai ridicate de poliploidie. Biometria exemplarelor de ierbar a relevat diferenţe microevolutive între populaţiile estice /din Moldova/ şi cele vestice. [illegible] microevoluţie a fost activată şi prin luarea ei în cultură la începutul secolului al XIX-lea, respectiv începerea lucrărilor de ameliorare în scop furajer /Elveţia, 1888/. În cultură s-au [illegible] populaţiile de tip latifolium cu caractere [illegible] valoroase din punct de vedere agricol: [illegible], raport ridicat de frunze, otăvire mai rapidă etc. [illegible] convarietate - convar. foliosum - [illegible] perene de talie înaltă de ex. Festuca arundinacea - [illegible] poliploidă, [illegible] ecologice, [illegible] etc.

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