Goals and results in improvement of biological background of medicinal plant production

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Summary: The choice of varieties among medicinal plant species is relatively small, compared to other horticultural crops. In Hungary, only poppy *(Papaver somniferum)* and mustard *(Sinapis alba)* have several cultivars. Recognising the problem, in the recent years breeding activity has been intensified all over the world, in spite of financial, technical and legal difficulties. The article reports on the results of breeding at the Department of Medicinal and Aromatic Plants of the Szent István University, 11 varieties of which has been officially registered till 2000. Main goals of genetic improvement are: increasing of the production capacity of utilised plant organs, enhancement of active material accumulation capacity, improvement of sensory quality and technological properties. The most often applied methods are selection, and recently, cross breeding, the results of which can be measured on new materials of caraway *(Carum carvi)*, hyssop *(Hyssopus officinalis)*, marjoram *(Majorana hortensis)*, poppy *(Papaver somniferum)*, etc. Efficacy of breeding work is established by additional, regular research on the genetics, physiology, floral and reproductive biology, chemosyndromes of medicinal plant species.

Introduction

Recent tendencies in making a more strict control on quality of medicinal plant drugs, phytopharmaceuticals and their other products calls the attention on quality assurance already from the start of the cultivation. Although several agrotechnical factors impact a considerable influence on the quality of the harvested product, the improved genetic material must be the base of the cultivation. The GAP Guidelines mentions among the first definitions the requirement of the documented origin of used plant material (*Franz & Máthé*, 1997). The well defined propagation material should be any kind of plant taxa, however in optimal case it means a selected cultivar.

In Hungary, the majority of materials resulting from breeding had been registered as official cultivars. Assurance of breeder rights by patent application is only usual for crops, which are utilised by the pharmaceutical industry in big quantities, like poppy or ergot. In 2000, 76 registrated cultivars had been known especially for medicinal and spice plant species (National List of Plant Varieties). Among them, a real supply exists only in case of poppy (*Papaver somniferum*), which possess altogether 11 varieties (*Table 1.*). Some other species (fennel, caraway, basil etc.) have a medium large spectrum of varieties, while there are 19 species which has only a single registered cultivar, among them savory, anis, milk thistle, lemon balm, etc. However, for the majority of species only populations of unknown origin or mixed materials are propagated and produced.

Table 1. Number of the officially registrated cultivars in Hungary (according to the National List of Varieties, 2000)

Nr. of varieties	Species
1	*Amsonia tabernaemontana, Anethum graveolens, Angelica archangelica, Artemisia annua, Cotynus coggygria, Echinacea purpurea, Hyssopus officinalis, Lavandula angustifolia, Leonorus cardiaca, Leuzea carthamoides, Levisticum officinale, Malva sylvestris ssp. Mauritiana, Melissa officinalis, Papaver bracteatum, Pimpinella anisum, Rosmarinus officinalis, Santolina chamaecyparissus, Satureja hortensis, Satureja montana, Silybum marianum, Verbascum phlomoides*
2	*Artemisia dracunculus, Brassica juncea, Calendula officinalis, Coriandrum sativum, Digitalis lanata, Matricaria chamomilla, Mentha x piperita, Majorana hortensis, Salvia sclarea, Secale cereale*
3	*Carthamus tinctorius, Carum carvi, Foeniculum vulgare, Ocimum basilicum*
5	*Cucurbita pepo var. styriaca*
7	*Sinapis alba*
11	*Papaver somniferum*

The situation reflect a poor supply of cultivars, compared with other horticultural species, and not only with those ones of high economical significance (e.g. tomato, cucumber) but even with species of a moderate turnover (e.g. egg-plant, celery, etc.). Reasons of the present situation may be explained by several factors:

– till the last decade, production and processing technologies did not really required plant raw of different characteristics and quality;

– variety spectrum could not be enriched either by foreign taxa because of the lack of them even in abroad;

– income from variety right ensures neither profitability nor a considerable background for future developments because of the relatively small areas and purchased seed lots.

The Hungarian variety spectrum may seem somewhat richer if compared with the variety supply of medicinal plant of other European countries. Regular and remarkable breeding activity and cultivar development is going on only in some countries, e.g. France, The Netherland, Italy, Germany. The activity in these countries and also in other ones is mainly focused on a restricted number of species (e.g. *Salvia* spp., *Origanum* spp., *Carum carvi, Chamomilla recutita*, etc.). As reasons, *Franz* (1996) mentions the relatively minor economical significance of these cultures as well as the insufficient regulation. Thus, comparing the supply of cultivars in Hungary and other states, we do not have a bad position. As an example, among the 23 varieties of caraway in the world, 3 are Hungarian ones. Neither of the countries possess more, than this (*Németh*, 1998).

However, considering the unique wide range of species and huge area of cultivation in Hungary, we may not resignate stopping cultivar development and effective breeding. An intensive breeding work has been going on in the last years mainly by the help of state supports. It resulted in considerable success. From the above mentioned 76 registrated cultivars 37 ones have been developed in the last 10 years. Each of them is Hungarian breeding, foreign cultivars are only found in case of *Sinapis alba*.

In Hungary, breeding activity of medicinal plants is going on in a relatively small number of institutions. One of the most active ones is the Department of Medicinal and Aromatic Plants at the Szent István University. Beside maintenance and improvement of formerly bred valuable cultivars, (*Carum carvi* 'SZK-1', *Foeniculum vulgare* 'Soroksári', *Matricaria chamomilla* 'Soroksári'), an intensive breeding work with a considerably widened spectrum of species is going on recently. Paralelly, special attention is paid to the genetical, physiological and technical questions, which -in lack of information- may slow down the breeding process. This manuscript is focused on the results in development of biological background of medicinal plants, achieved by us in the last years.

Increasing of the production capacity of utilised plant organs

Reaching economic yield of active materials, the genetic material is required to have an optimal biomass production as well as a proper level of active agents. Beside some special aims, these requirements are the most general goals in breeding of medicinal plants in our days.

Enhancement of biomass formation of the overground parts had been one of the focus points during the development of the new Hungarian marjoram (*Majorana hortensis* Mönch.) variety 'Magyar'. Selection for higher drug yield was carried out by examining plant height and width, plant habit, proportion of leaf and stem mass, leaf size (surface) and the mass ratio of crumbled/whole drug. The results demonstrated the positive correlation of the majority of these traits with the drug yield (unpublished data). Our data coincide with the findings of *Pank* et al. (1999), who proved a tight connection between drug yield and bush width while it correlated less with the leaf surface area. In the late 90ies, the variety tests of the National Institute for Agricultural Quality Control proved the success of our breeding efforts (*Table 2.*).

Similar results were achieved in cultivar trials with the peppermint variety 'Mexián' which had been registered in 1997. In comparison experiments of five materials, `Mexián` reached the highest yield (*Table 3.*). The measurements

Table 2. Results of the variety test examinations of the Hungarian National Institute for Varieties 1998-99

Variety	Yied				Essential oil content %	Essential oil yield kg/ha
	fresh shoots		dry herb			
	t/ha	%	t/ha	%		
'Magyar'	5.49	146	1.49	144	1.58	23.54
'Francia'	3.76	100	1.03	100	1.43	14.72

Table 3. Productional characteristics of the variety 'Mexian' compared with other materials (adapted from *Németh* et al., 1997/a)

Popula-tion	first year				second year			
	plant height (cm)	leaf lenght (cm)	leaf width (cm)	folium mass (g/m²)	plant height (cm)	leaf lenght (cm)	leaf width (cm)	folium mass (g/m²)
'Mexián'	54.0	7.46	3.46	69.0	65.5	8.90	3.50	439.0
Finnish	43.4	5.78	3.56	55.0	60.5	6.10	2.90	338.0
'Mitcham'	43.6	5.88	2.84	49.0	52.1	5.40	2.50	248.0
USA	45.3	5.36	3.10	47.0	53.1	4.90	2.40	250.0
Strain 10	47.2	5.68	3.26	55.0	55.5	5.40	2.90	286.0
LSD	8.46	1.02	0.33	9.5	6.52	0.79	0.34	114.0
p%	1.00	1.00	1.00	5.0	1.00	1.00	1.00	5.0

showed the role of the leaf size in determination of drug yield. The determination coefficient between leaf surface and *folium* yield proved to be 86-93% at summer cuttings and 63-67% at autumn cuttings. The correlation coefficient was 0.82-0.97, for each traits significant (*Németh* et al., 1997/a).

Especially in case of species assuring *herba* and *folium* drugs, the productivity of genotypes can be increased also by enhancement of the regeneration ability and yielding capacity of the newly shooted plants during the second cutting. Eventually, in the autumn harvest 'Mexián' proved to have a yield 59-64% of the main cutting, while some of the other origins reached a value of 71% in this respect (*Németh* et al., 1997/a). Recently, improvement of the new cultivar by clonal selection is carried out for increasing its regeneration capacity.

Selection for higher biomass production from populations of great intraspecific variability could be speed up by vegetative propagation. At the Experimental Station of the Department, accessories developed by clonal selection of *Origanum vulgare* ssp. *hirtum* had been compared in a four years trial. The examinations showed, that offspring populations exhibited different productional values (*Table 4.*). Yield differences especially for the mass of flowers

Table 4. Differences in clones of Origanum vulgare ssp. hirtum developed by clonal selection; 2nd year old stand 1997, (adapted from *Szabó*, 2001)

Feature	Clone H1	Clone H2	Clone H3	Clone H4	Clone H5
Height (cm)	52	54	53	61	55
Nr. of shoots	38	43	39	37	55
Dry shoot mass (g/plant)	37	34	23	54	62
Mass of flowers (g/plant)	9	7	5	13	19
Essential oil content (%d.w.)	4,9	6,6	2,2	2,8	4,8
Carvacrol in the oil (%)	90	75	36	42	88

reached a 3-4 fold one. The productional diversity proved to be stable during the experimental period, thus the best clones are promising materials for cultivar development for the practice (*Szabó*, 2001).

Changing of active material accumulation capacity

The genetic determination in reaching an optimal level of active agents in the drug, seems to be proved for a great number of chemical compounds (e.g. *Hefendehl* & *Murray*, 1975; *Nyman*, 1980; *Franz* & *Wickel*, 1985); *Vernet* et al., 1986). Therefore the genetic background assures the bases of optimal quality of products, even if several other factors (ecological circumstances, agrotechnology, processing, etc.) may have an influence on manifestation of the genotype.

The up-to-date drug specifications (pharmacopoeia, standards, etc.) regulate both the content as well as the composition of the ingredients. To reach these quality requirements, the established cultivars should possess a firm genetic base for optimal and stable accumulation of the desired active materials. From this point of view, genetic improvement at the Department in the last years has been oriented on plants containing essential oils and alkaloids.

Most often, the breeding aims are focused on enhancement of the accumulation level of the desired active components. As an example, the variety 'Soroksári' of fennel has been selected for high essential oil content in its fruits and registered in the early 90ies. Since then, comparison trials proved the significantly higher essential oil accumulation capacity of this cultivar against the huge majority of other origins (*Figure 1.*). The essential oil level

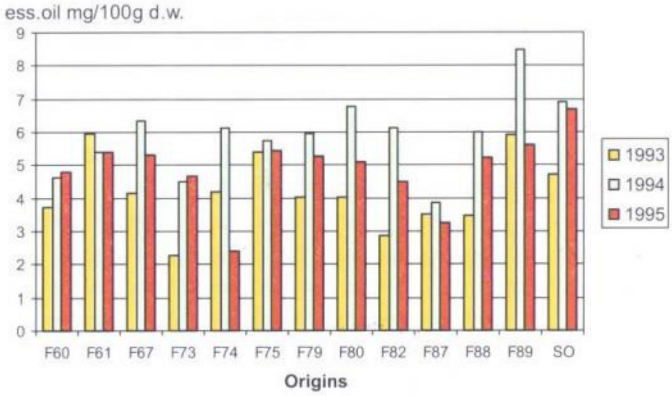

Fig. 1 Essential oil content of the examined fennel *(Foeniculum vulgare Mill.)* origins during 1993–95, including SO= variety 'Soroksári' (according to Kattaa, 1996)

reaches or exceeds 5%, in advantageous years even 7% in the ripen fruits. Main component of the oil is anethole in 67–74% (*Kattaa*, 1996).

Intensive selection work has been carried out also on other species containing essential oil. Aims of breeding of hyssop (*Hyssopus officinalis* L.) were reaching a high essential oil content of the drug (above 1% d.w.), besides, producing a population uniform in flowering time and possessing dark blue coloured petals. Starting from a population of our genebank collection (mean essential oil content 0.8 %), during the last seven years a considerable increase of the essential oil content of the drug has been achieved (*Figure 2.*). Although the actual contents of the drug had been influenced also by weather conditions, especially by the amount of precipitation (*Németh* et al., 2001/a), the accumulation capacity seems to be stabilised around a level of 1,1-1,2% d.w as result of the selection.

Recently, in some special cases, the decrease of accumulation level or elimination of certain components in the drug may come into the limelight of breeding. In case of *Salvia officinalis* L. the future aim, -together with word

Fig. 2. Increase of essential oil content in *Hyssopi herba* as result of the yearlong selection

tendencies- is the development of a chemotype of low level of β-thujone in the essential oil (*Bernáth & Németh*, 2000). In our gene bank collection, examination of 40 individuals during two years and four cuttings showed a significant variation of thujone level in the oil which may serve as base for successful selection (*Table 5.*). It was shown, that the

Table 5. Composition of the essential oil of *Salvia officinalis* individuals in gene bank collection of the SZIE (June 1999, Soroksár; unpublished results)

Component	Mean	Minimum	Maximum	CV%
a-pinene	1,5	0,1	5,8	81,2
camphene	3,5	0,5	6,1	37,2
b- pinene	2,5	1,5	3,8	25,9
limonen	8,9	2,5	14,7	29,7
a- thujone	34,1	17,7	50,5	24,9
b- thujone	7,8	2,7	23,2	83,7
camphor	22,8	3,0	34,6	25,9
borneole	2,5	0,4	17,3	116,0
β- cariophyllene	1,8	0,5	3,8	53,4
β- selinene	4,2	2,0	9,7	37,5
ledol	5,7	3,5	8,2	22,6

contents of α- and β-thujones are in a strong, positive, linear correlation with each other (r=0.64-0.75), (unpublished data).

Poppy is an alkaloid containing species representing a special topic. Here, breeding has been carried out in different directions parallel (*Bernáth & Németh*, 1999):

– creating cultivars accumulating high morphine (1.5–2.0%) or special alkaloid content (narcotine, codeine, thebaine etc.) for industrial extraction;

– selection of cultivars for seed and oil production, accumulating low level of alkaloids in the capsules (0.01% or less);

– producing ornamental types with special flower or capsule form, accumulating restricted amount of alkaloids.

In each breeding directions significant achievements could be registered in the last years. Among the high alkaloid types, new cultivars, containing special alkaloid spectrum had been developed, using also the mean of environmental pressure under controlled phytotron conditions. As a result, the Hungarian spectrum of poppy varieties has been extended and today alkaloid patterns are well defined characteristics in identifying several varieties (*Figure 3.*).

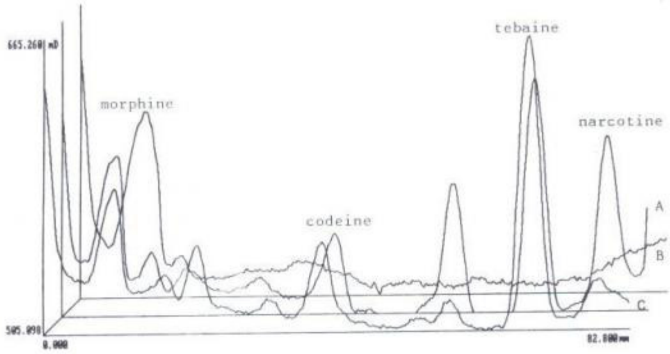

Fig. 3. Densitogram of three Hungarian poppy varieties showing characteristic alkaloid patterns
A - 'Kék Gemona' B - 'A1' C - 'Tebona'

Breeding for low alkaloid content has been started three years ago. After evaluation of a wide collection of poppy origins, new strains had been developed by reciprocal crossings of the best materials and followed by individual selection. The majority of F1 strains did not proved to be uniform but homogeneity of the strains increased during the breeding process. In F2 and F3 generations, the selected lines of common origin (parental combination) were well distinguishable both from each other as well as from their parents. From the point of view of the main aim of breeding, three of the six basic parental combinations gave successful results, in some of them, even the maximum values within the strain do not reach a morphine content of 0.01 %o. Contents of codeine, tebaine and narcotine are below the detection level (*Németh* et al., 2001/b).

For assuring the supply of poppy cultivars of decorative value, the variety 'Óriás kék' has been developed and registered in 1998 both as medicinal as well as ornamental plant cultivar. It possess an interesting red colour of flowers and big, attractive capsules which suit well for dried flower compositions (*Figure 4.*).

Fig. 4. *Papaver somniferum* 'Óriás kék'

In evaluation of new materials, comparison trials of poppy, existence of official DUS test guidelines assure a considerable advantage. It has been developed by Hungarian specialists in the late 90ies (*Köck*, 1998) and till now the only one among medicinal plant species which had been accepted internationally.

As for other species, activity at the Department include development of appropriate comparison methods, too. Recently, in case of hyssop, investigation has been carried out for determination of group of the most suitable plant characteristics in evaluation of different origins. Based on measurements in 5 populations, during 3 years, we described 11 plant characteristics which included: plant height, earliness, thichness of bush, branching, length of internodes, colour, length and width of leaves, anthociane colouring, petal colour, essential oil content. Using them as discriminative factors, the experimental populations were well distinguishable form each other (*Figure 5.).* It was proved during the

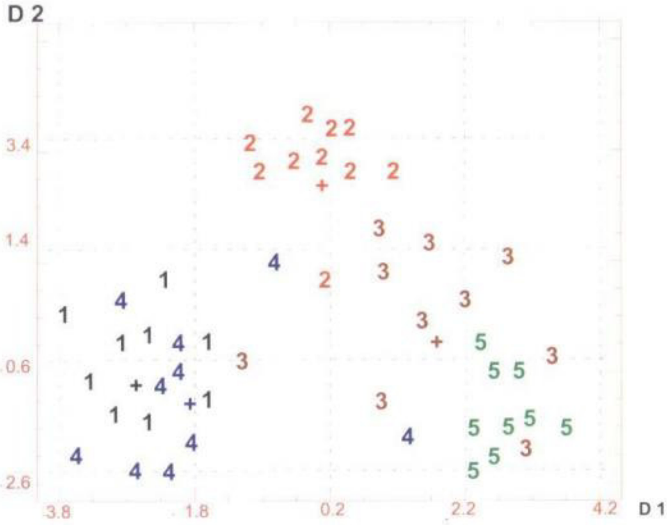

Fig. 5. Distribution of populations of *Hyssopus officinalis* based on 11 appropriate traits (Soroksár, 1998)
D 1: Discriminant function 1; containing plant height, petal colour, strength of branching, essential oil content
D 2: Discriminant function 2; containing length of leaves, colour of leaves, anthociane colouring of shoots

calculations, that leaving out three of this traits (height, thickness, length of internodes) does not influence the efficacy of distinction, however falling out some further ones gives unfavourable results (unpublished results).

Supporting the breeding activity for changed active material content and composition, a special attention is paid to determine distinction between the genetically fixed chemism and its modifications, called chemosyndromes. The most important influencing factors in evaluating chemical features include the sampling time during the vegetation period (phenological phase), organic differentiation of plants, weather conditions, effects of year or age of plant individuals, extraction or analytical methods. Similarly to the essential oil of hyssop, considerable influence of the year was proved for the active material content of *Hypericum perforatum* It should be taken into consideration both for hypericin as well as for the total flavonoid content of the shoots (*Pluhár* et al., 2000). Results are similar for alkaloids: the weather conditions, especially high precipitation level makes the distinction of poppy cultivars on base of their characteristic chemical components difficult. It is especially pronounced in case of methylated compounds, such as codeine and tebaine (*Bernáth* et al., 1999).

In the middle of the nineties we adapted a valuable method for reliable extraction of original terpenoid compounds of drugs. In case of marjoram as model species, derivatives of sabinene, suffered considerable transformations during extraction by distillation (*Figure 6.*). The new supercritical CO_2 extraction (40°C, 120 bar, 30 min.) enables characterisation and effective selection of the best individuals according to the parameters of the intact plant (*Pluhár* et al., 1996).

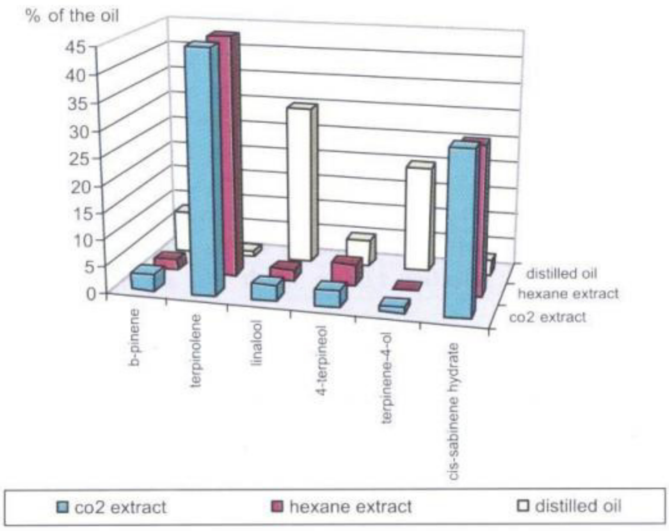

Fig. 6. Composition of marjoram oil produced by different extraction methods (*Pluhár* et al., 1996).

Improvement of sensory quality

Appropriate drug quality presumes appropriate sensory characteristics as well. It is even more important in case of raw for alimentary and cosmetic industry (spices, condiments,

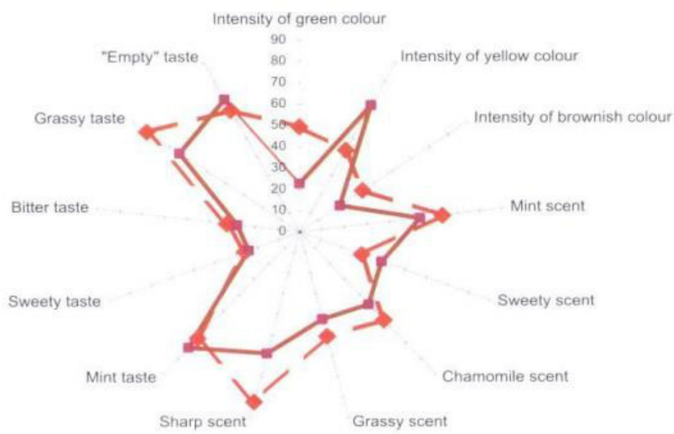

Fig. 7. Profile diagram of the sensory evaluation of peppermint drugs from cultivars 'Mexian' and 'Mitcham' (*Németh* et al, 1997)

aromas). While medicinal plants as pharmaceuticals should be characterised by a stable ingredients' spectrum defined by chemical analysis, products of the mentioned utilisation areas have to face with sensory analysis. For species of multiple utilisation possibilities, new cultivars desirably should show a stable and distinguishable sensory profile.

Examinations at our university proved, that the variety 'Mexián' of *Mentha piperita* L. fulfil these requirements. In profile analytical sensory tests, for 4 characteristics among the 13 evaluated ones, significant differences could be proved between the new variety and the 'Mitcham' one (*Figure 7.*). The characteristics appropriate for distinction included various colour and odour features (unpublished data). Divergences in the organoleptic characteristics of this variety from other mint origins were proved also by instrumental sensory trials with the help of the „electronic nose" (*Novák* et al., 2001).

Improvement of technological properties

From growers' side, plant characteristics which enable special agrotechnical procedures have outraging importance. They may be connected with culture duration (annual-perennial), growth intensity (earliness), competition ability (weed tolerance), propagation specialities (closed fruits, uniform germination) or plant morphology (size of organs, growth habit, bush form), etc.

Today there is a world-wide lack in medicinal plant cultivars which had been selected for special technology and in consequence, assure different production values for the farmers. Poppy may be an exception also in this respect. Under Hungarian conditions, spring and winter varieties represent not only agrotechnical differences but are also the genetic base for different production capacities. Widening the spectrum of winter sown poppy varieties of appropriate alkaloid content would increase safety of production and assure a flexible crop rotation. Works in this direction has been started recently (*Petheő* et al., 2001).

In genetic improvement of caraway, goal of breeding has been the combination of first year flowering of the annual form and high essential oil and carvone level of the biennial one. As additional aims, uniform ripening, high yield and lack of residues of carpophores on the fruits had been set. Reciprocal crossing of f. *annua* and f. *biennis* of *Carum carvi* L. has been carried out in 1995 and the hybrid progenies tested and selected during the following period. In F2 populations, progenies of the annual and those of the biennial mother plants exhibited significant differences in flowering proportion (*Németh* & *Pluhár*., 1996). In consequence of the selection, the F6 progenies became uniform in flowering (*Figure 8.*), besides, assure a stable essential oil content above 3,5% with a carvone proportion of 52–56%. This hybrid strain is before the official application for variety registration.

Reaching the mentioned results arised the need for additional investigations on reproduction and floral biology

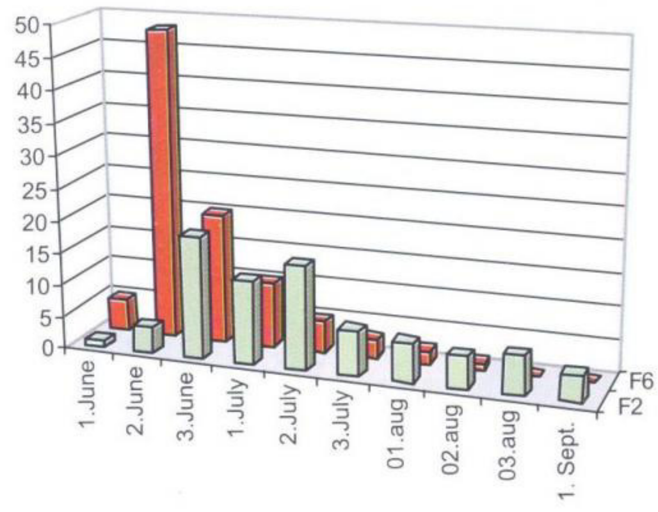

Fig. 8. Flowering dynamics of crossed caraway strains in F2 and F6 progenies.

(flower development, compatibility, fertilisation, etc.) which represent less known and hardly searched areas for medicinal plants. At the Department studies in this respect include several species. Special attention is paid to the *Apiaceae* and *Lamiaceae* species. Results of ecological examination on flowering induction of biennial caraway assured the technical base of cultivar development by crossing in case of caraway (*Németh* et al., 1997/b). Similarly, observations on flowering dynamics and fertilisation properties of fennel and caraway assure the optimal maintenance of varieties and selected strains, determination of phenophases during isolation and harvest, sampling in chemotaxonomic studies, etc (*Németh* et al., 1999). Determination of a new flowering index formula proved to be an accurate model for the description of flowering process in basil. Beside its utilisation in breeding, even the accumulation process of the essential oil could be characterised by the flowering index values, because of their close correlation (r=0,96), (*Szabó* et al., 1999).

One of the main goals of breeding is the improvement of technological properties also during introduction of wild growing species into the agriculture. Development of growing methods is going on parallel with establishing suitable genotypes for economic cultivation.

Spectacular result in the last years was the development of variety 'Napfény' of *Verbascum phlomoides* (*Figure 9.*). This cultivar possess the capacity to develop flowers already in the year of sowing. Although the individual yield does not reach that of the wild growing biennial genotypes, production calculated for two vegetation cycles and unit area assures profitable production of this species in agrar-ecosystems in annual crop rotation (*Szépréthy* & *Németh*, 1995). Content of flavonoids of the drug is practically equal with that of the wild material and exceeds the requirements of PhHg.VII. (*Table 6.*).

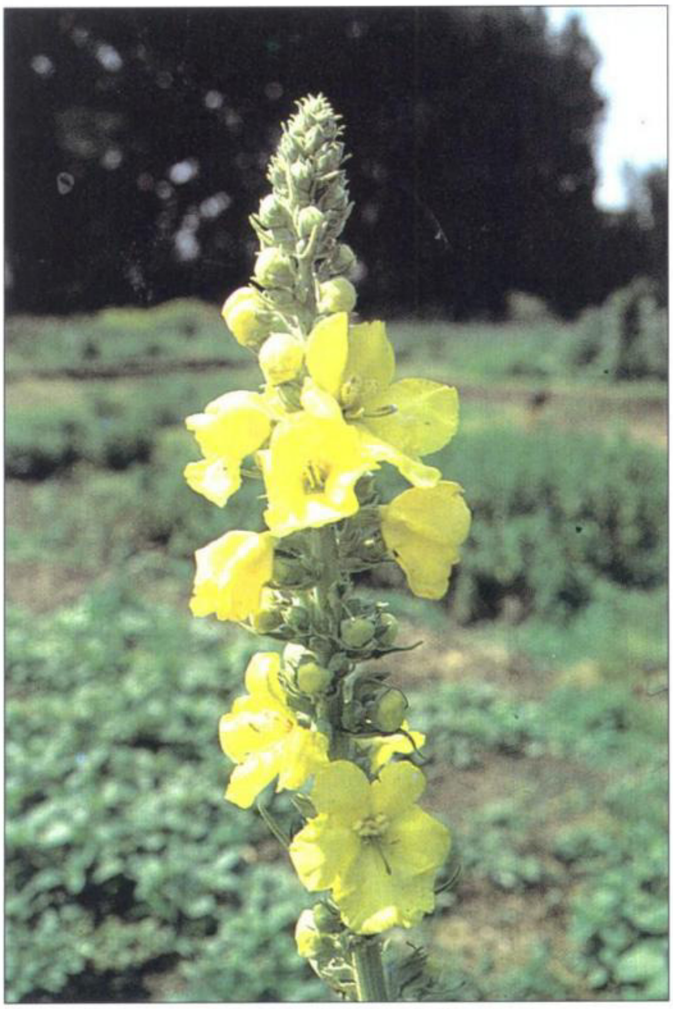

Fig. 9. Verbascum phlomoides 'Napfény' (Sunshine)

Table 6. Production values and flavonoid content of variety 'Napfény' and the biennial control population (adapted from *Szépréthy & Németh*, 1995).

Genotype	Nr. of side-branches of the shoot	Nr. of flowers/plant	Diameter of flowers (cm)	Total flavonoid (% d.w.)
'Napfény' -annual	3,5	213	4,32	1,04-1,30
Wild type -biennial	14,6	1096	4,53	1,01-1,12

Introduction of not indigenous species into the Hungarian agriculture may cause special problems. First of all, ecological factors should be taken into consideration. In these cases, goal of the genetic development is the increase of adaptation ability of the genotypes for our conditions. Two variety registration from the 90ies show the success of these work at the Department. The variety 'Indián' is a non-sensitive, high yielding, perennial taxon of the North-American species *Echinacea purpurea* (L.) Mönch. The cultivar 'Harmat' of the Mediterranean species *Rosmarinus officinalis* (L.) has been selected for frost tolerance. It shows also a special chemical character having the monoterpene compounds 3-octanone and verbenone not described before in the essential oils of rosemary (*Domokos* et al., 1997).

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