Investigation of flowering dynamics of the basil *(Ocimum basilicum* L.) and its consequences in production

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Summary: The flowering dynamics of *Ocimum basilicum* L., (a common population maintained at the UHFI) was studied with the aim to create an exact and practically applicable method for definition of phenological phases.

According to our observation the development of individual flowers of the basil can be characterized by 8 distinguishable phases, which must be considered for description of the actual phenological stage of a spike.

An accurate model was created for the unambiguous description of flowering process of different flowers within the spike and for the individual plant as a whole. The new flowering index formula is calculated from the number of flowers weighed by their phenological phases.

The time dependence of flowering is presented by functions fitted to values of the flowering index. The results reflected different patterns of the main inflorescence, the inflorescences formed on the side shoots in the first or in the second half of the flowering period. However, for description of the flowering process of the whole plant, a sigmoid function proved to be the appropriate model.

The accumulation process of the essential oil could be characterized by the flowering index values. They showed close correlation (r=0.964) at high probability.

The new method assures an exact definition of phenological phases in basil. Its application seems to be optimal during breeding procedures, seed production and even in production of high quality drugs, according to the requirements of the Good Agricultural Practice (Guidelines for GAP, 1998).

Introduction

Ocimum basilicum is an annual medicinal and aromatic plant from Lamiaceae family. Its main active agent is the essential oil accumulating in the aboveground parts of the plant in 0.5–1.5 per cent. The high-scale utilization of the drug produced from the species is due to its favourable dietary and seasoning effect. Basil is cultivated in higher volume in Spain, Turkey, Italy, Egypt, Pakistan, Bulgaria and Hungary *(Lenchés* in *Bernáth,* 1993).

Large number of publications dealt with the floral biology of the plants, including its botanical, physiological, genetical or production-biological aspects. For instance morphological characters of 17 cultivars of different *Ocimum* species were examined by *Moreno* et al. (1987). On the basis of their investigation, the presence of the diverse types of the compound inflorescence as well as the high morphological variety of the individual flowers was described. Making pollination tests, it was stated by *Nation* et al. (1992) that the slight outcrossing between different *Ocimum* cultivars could be the consequence of the high morphological variability of perianth. Similarly, the crossing among *Ocimum* taxa were applied by *Paton* and *Putievsky* (1996) as well as *Ryding* (1994) to explore the degree of genetic relation among of plant materials of different origin.

Based on the above mentioned publications, it is obvious that the knowledge of macro- and micromorphological characters of generative organs, the floral biology and flowering dynamics of the individual species, subspecies are essential ones for both the practical implementation of crossing and the taxonomical evaluation of hybrids. A similar consideration was accepted by *Németh* et al. (1998) exploiting the floral biology of *Carum carvi* and *Foeniculum vulgare*. The probability of the outcrossing of each species was identified by them gaining both theoretical and practical results concerning flowering dynamics of the species.

Besides the above mentioned studies on flower biology, numerous production-biological studies are known trying to identify the proper phenological phase of sampling. *Putievsky* (1993) studied the ripening process of seeds, depending on their position both inside the individual inflorescence, as well as on the position of the inflorescence on the plant.

During the growing season quantitative and qualitative changes of active ingredients were identified in the drug by *Lemberkovics* et al. (1993, 1996). In this work, the generative phases of the plant were determined as follows:

early flowering, full flowering, late flowering, early ripening and ripening stages. The accumulation of main groups of essential oil compounds (monoterpenes, sesquiterpenes) and that of individual compounds, as well as the presence of flavonoid glycosides and free flavon-aglycones were detected in relation to these phenological stages. According to their results the highest essential oil content was measured in the early flowering stage.

In another study, the essential oil content reached its maximum value at the stage defined as complete flowering (*Randhawa* and *Gill,* 1995). The fresh yield, the height, the number of branches and the essential oil yield increased up to the same development stage as well. The decrease of cineole and increase of linalool were observed parallel with the development of plants.

Three different development stages of harvesting (ie. early, full and late flowering) were distinguished by *Basker* and *Putievsky* (1978), in which the quantity of essential oil and the production of dry leaves were determined. Involving different cultivars, much more, 8 different terms of harvesting were managed by *Gupta* (1996), studying the quantity of herb, the essential oil content and its composition. The terms of sampling were characterized by the following phenological phases:

- seedling stage (60 days after sowing)
- pre-flowering stage (90 days)
- 50% flowering stage (120 days)
- 100% flowering stage (150 days, maximum of herb yield)
- initiation of seed set stage (180 days)
- 50% seed set stage (210 days, maximum of methyl-cavicole)
- seed ripening/maturation stage (240 days)
- seed maturation stage for late maturing lines (270 days)

The correlation between the essential oil accumulation and plant development was proved by *Mihalik* (1992) by histological studies. According to her results, the accumulation of terpenoid components starts to intensify at the time of the flower-bud development. During the flowering and early fruiting period the continuous increase of size and number of lipophilic droplets in the leaves was observed.

In the mentioned studies, the lack of exact definition of phenological phases decreases the reproducibility of samplings, the possibility of comparison of investigations and it produces in some cases contradictory results. It seems to be necessary to gain proper data on the flowering process, to be able to define each phase correctly. Those informations are the condition of drawing further consequences in connection with seed setting, essential oil accumulation etc. can be drawn. Further on, knowledge on the flowering characteristics is a prerequisite for successful breeding.

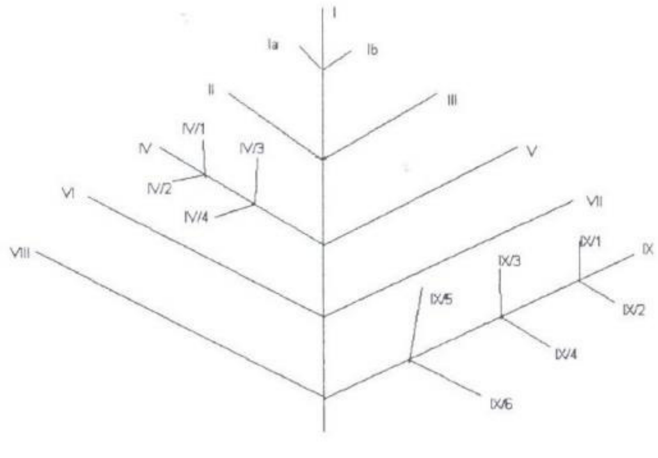
In the recent study, our intention was to characterize and describe the flowering dynamics of basil, giving numerical definition of the flowering stages. Furthermore, based on the proper numerical definition of the stages a quasi model on the development and essential oil accumulation was built up.

Material and methods

The open-field experiment was carried out in Soroksár, at the Experimental Station of the Department of Medicinal Plant Production, University of Horticulture and Food Industry.

In the experiment the population of *Ocimum basilicum* cv.'A-1' was used. The plant material, which was selected at the Department can be characterized by adequate morphological and chemical homogeneity. The plant material was grown up by sowing seeds on 25th of April in greenhouse, pricking on 12th of May and planting out on the 28th of May. The experimental population consisted of 60 individual plants with spacing at 60x40 cm. During the growing season the plants were irrigated regularly.

The study in floral biology was carried out from the beginning to the end of flowering period (between 17 July and 05 September). Measurements were made every 3 or 7 days (altogether 18 times) depending on the intensity of blooming, on three representative individuals of the population. Developmental phases of flowers were registered within the inflorescence, continuously. For the proper identification, the apical inflorescence and those the ones formed on the side-shoots were marked individually and ranked according to their position *(Fig.1)*.



I: main inflorescence
Ia, Ib: side-shoots of main inflorescence
II–IX etc.: side-shoots
II/1–IX/6 etc.: Secondary branches of the side-shoots (On the picture are two examples)

Figure 1 Codes of inflorescences and of side-shoots of basil

For an accurate model to describe the simultaneous changes of different flowers within the spike and in the case of the individual plants as a whole, the numerical definition of flowering of *Adonis vernalis* was taken into consideration *(Máthé* 1977). His special flowering index formula given below was checked in our experiments and used for further calculations.

$$\frac{\text{number of fading flowers} - \text{number of buds}}{\text{number of all flowers}}$$

For checking the essential oil accumulation during the development, plants were harvested in 8 cases from the above mentioned 18 flowering measurements. The samples taken from 3 average individuals each time, were dried and crushed. The distillation of essential oil was made by Clevenger apparatus in the laboratory of the Department using 10 g drug and expressed in % of the dry mass.

For the mathematical evaluation SPSS statistical program was used. For modelling the time dependence of flowering, fitting function was applied, which was checked by the help of correlation coefficients. To describe the correlation between the essential oil content and the numerically defined phenological stages, correlation analysis was used as well.

Results

The flowering stages of each single flowers of basil show the general characteristics known for *Labiatae* species. According to the fundamental interpretations **the lifetime of a flower** is starting with the appearance of the bud, which is followed by flowering, fertilization, the collateral wilting process and ageing. Nevertheless, during studying **the lifetime of any kind of inflorescence,** the flowering period must be defined differently from the fundamental interpretations of individual organs. The flowers within the basil's spike open continuously, parallelly with the development of the spike. For this reason the description of the actual phenological stage of inflorescence is a complex one and for its proper definition the development of each single flower must be considered.

According to our observation the development of individual flowers can be characterized by the following phases:

- green bud stage: the whorl has already separated on the axis of inflorescence, the petals are greenish, and from side-view the calyx still covers the corolla,
- white bud stage: the petals are white and turgid, from side-view the calyx does not cover them,
- opening of the flower: the petals just before opening, or the stigma and stamina are already visible, however, the corolla is still tubulous,
- fully open flower: the typical *Labiatae* corolla appears, the upper and lower lips diverges from one another, stigma and stamina show their characteristic colour,
- fading of the flower: the stigma and stamen become brown, the petals show some kind of wilting,
- white seed stage: from the abscission of petals to the development of full size of schizocarps
- brown seed stage: the colours of fruits show the different shade of brown, their ripening began
- black, ripe seed stage: characteristic colour and size of the schizocarps appear

In contrast to the former descriptions, the flowering stages observed are much more detailed, consisting of 8 different stages. According to our observation budding stage has to be divided into two different stages (green and white buds) and even after the flowering 4 further developmental stages can be separated. Because of the importance of these special developmental stages, in the case of basil determining the developmental stage of the individual spikes and the plant as a whole, the flowering index of *Máthé* (1977) had to be modified. The new index formula was calculated by weighting the number of flowers by their correct developmental phase. The weights built into the formula are as follows:

Description of developmental stage	Code of the stage in the flowering index formula
A: green bud stage (bud stage)	1
B: white bud stage (bud stage)	2
C: opening of flower	3
D: fully open flower	4
E: fading of the flower (fading stage)	5
F: white seed stage (fading stage)	6
G: brown seed stage (fading stage)	7
H: black, ripe seed stage (fading stage)	8

Thus the flowering index formula modified for the exact determination of the development stage of basil inflorescence is as follows:

$$\frac{\sum(\text{number of flowers in stages E...H X code of E...H}) - \sum(\text{number of buds in stages A.B X code of A.B})}{\sum(\text{number of flowers in stages A...H X code of A...H})}$$

The value of flowering index calculated by this formula varies between number –1 and +1, theoretically. At the presence of budding stages only, its value is –1, and increases continuously afterwards by the appearance of more advanced stages. The flowering index value calculated by the codes of all flowering stages facilitates an unambiguous definition of the spike.

These flowering index values could be calculated for the main inflorescences, its branches, and for each side-shoot. The results are shown in *Table 1*. The values reflect the fact, that flowering of basil plant is going on in a defined order. The main inflorescence of the plant develops on the main axis, which is followed by the development of side-shoots, starting to grow after each-other in basipetal order. During the growing season the shoot formation and flowering order starting at the top can be observed on each side-shoot, too. It can be observed, that the index value of -1 lasts for several days, generally 2-5 days. The value never reached +1 because of the slowing down of plant development in autumn and its stop by the first frosts. From the data it is also obvious, that earlier flowering results in earlier ripening.

The time dependence of flowering is presented by functions fitted to the flowering index values. The results reflected two different behaviours. Sigmoid function could be fitted to the values calculated for the *main inflorescence* and *inflorescences formed on the side shoots* which started their flowering *in the first half of the flowering period* (until 25–30 of July) *(Fig. 2)*. In contrast, the equations calculated for the flowering index of *side-shoots appeared in the second half of generative phase,* can be characterized by an irregular course. Each of the linear, quadratic, or cubic curves can be fitted to them only with low probability (**r** <0.6) *(Fig. 3)*. In a few cases within a short period (approx. 20 days) the curves show a characteristic and abrupt drop *(Fig.4)*. It can be explained by the sudden opening of several flowers on the same inflorescence, causing a decrease of the flowering index. The physiological background of this phenomenon is still unclear. However, the changes going on the inflorescence developed in the second half of the generative period do not affect characteristics of the whole plant, because of the restricted number of those late coming flowers.

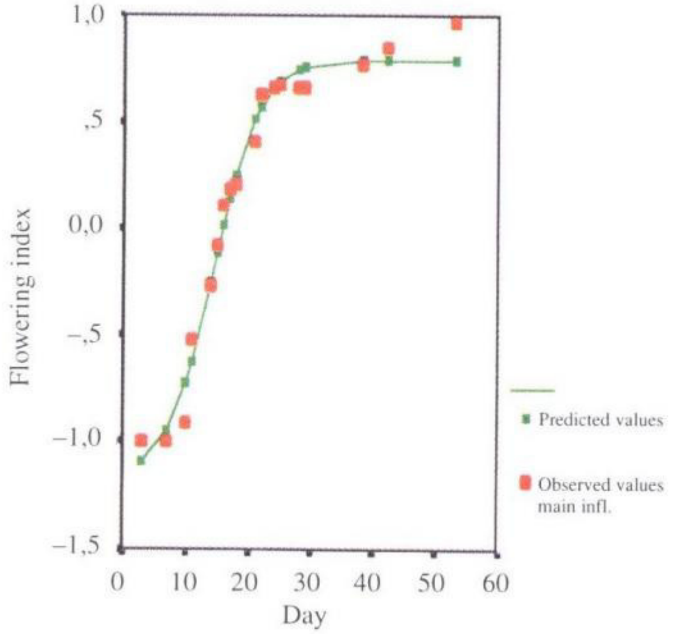


Figure 2 Mathematical modelling of flowering index values by fitting a sigmoid function

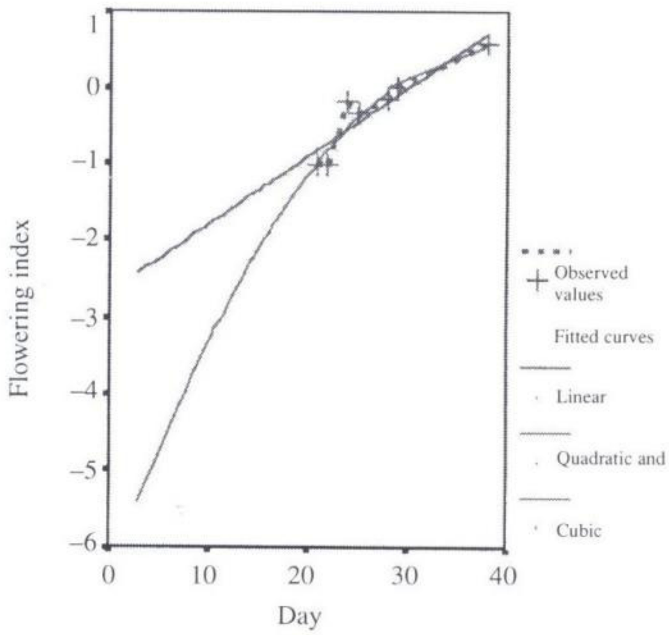


Figure 4 Function fitting on flowering index of side-shoot VIII.

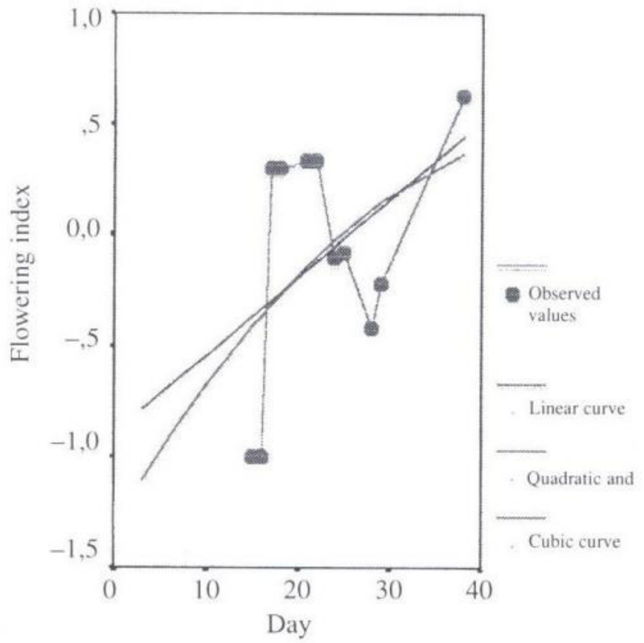


Figure 3 Function fitting on flowering index of side-shoot XV.

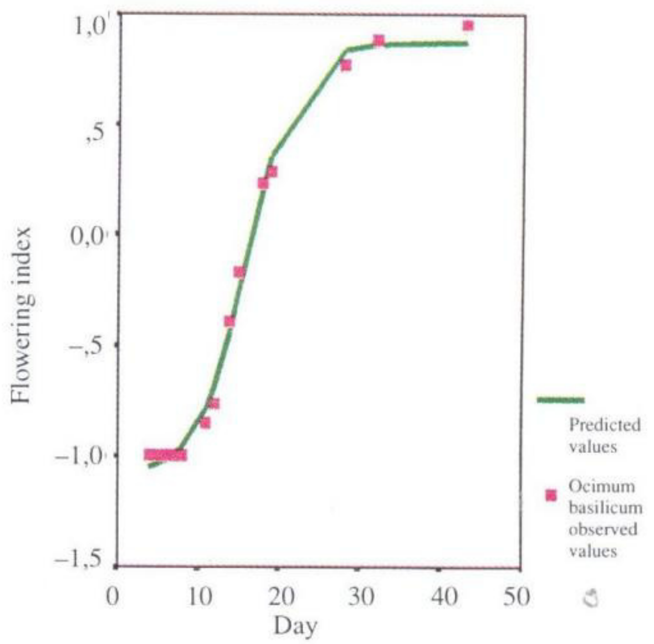


Figure 5 Sigmoid function of the flowering index of *Ocimum basilicum* individual

Taking into consideration all flowering index values of *every inflorescence* formed on a single plant (main inflorescence and inflorescences formed on side-shoots of different ranks) sigmoid function could be fitted to the joint index values of any individual of *Ocimum basilicum* cv.'A-E(*Fig. 5*). The equation calculated is the following:

$$Y= P1+(P2-P1)/(1+e^{-P3*(X-P4)})$$

P1 is the minimum value of the equation (= **–1**); **P2** is the maximum point, equal to **+1**; **P3** is the slope of the curve, which takes a value of about **0.2**. **P4** is the inflexion point referring to the time when the dinamics of flowering is slowing down. This point occurs on the 20–27th day counted from the beginning of the flowering.

characterize the sigmoid curves in the majority of cases. Thus, according to the statistical parameters of regression analysis, the curves of flowering index of side-shoots developing in the first half of the generative period, represent the flowering index of the whole plant properly. It means, that for practical application and definition of the phenological phase of a plant, measurements on the first side-shoots and calculation of the flowering index according to them, may be sufficient.

The described method of developing the formula of flowering index allows to express the flowering stage of basil individuals in any actual moment, properly, although in the future its extension to different types of cultivars is necessary.

According to our results, the flowering index could be

Table 1 Average values of the flowering index of the main inflorescence and of the side-shoots, first individual (Soroksár, 1997)

date	I	Ia	Ib	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
1997.07.17	-1														
1997.07.21	-1														
1997.07.24	-0.91														
1997.07.25	-0.53														
1997.07.28	-0.27	-1	-1												
1997.07.29	-0.08	-1	-1	-1											
1997.07.30	0.107	-1	-1	-1		-1			-1						
1997.07.31	0.179	-1	-1	-1		-1		-1	-1			-1			
1997.08.01	0.207	-1	-1	-1	-1	-1	-1	-1	-1		-1	-1			
1997.08.04	0.494	-0.854	-0.727	-0.759	-1	-0.914	-1	-0.893	0	-1	-1	-0.667	-1		
1997.08.05	0.627	-0.765	-0.563	-0.568	-1	-0.549	-1	-0.743	0	-1	-1	-0.704	-1		
1997.08.07	0.658	-0.396	-0.101	-0.094	0.135	0.031	-0.294	-0.02	-0.512	-0.424	-0.864	-0.677	-0.787		
1997.08.08	0.669	-0.173	0.173	-0.008	0.18	0.132	-0.048	0.059	-0.473	0.084	-0.483	-0.42	-0.455		
1997.08.11	0.652	0.237	0.178	0.222	0.226	0.187	0.303	0.274	-0.354	0.048	-0.206	-0.327	-0.148		
1997.08.12	0.661	0.287	0.237	0.297	0.319	0.21	0.38	0.39	-0.255	0.101	-0.169	-0.157	-0.062		
1997.08.21	0.765	0.765	0.736	0.626	0.701	0.585	0.459	0.496	0.424	0.406	0.569	0.442	0.687	0.744	0.244
1997.08.25	0.846	0.878	0.845	0.652	0.821	0.641	0.683	0.648	0.569	0.625	0.561	0.671	0.715	0.595	0.223
1997.09.05	0.964	0.947	0.948	0.892	0.97	0.879	0.827	0.839	0.848	0.815	0.856	0.87	0.856	0.782	0.765

The curves describing the behaviour of the whole plant and that of side-shoots appearing in the first half of the flowering period in majority of cases are equal at a probability of 95 % (*Table 2*). The same parameters

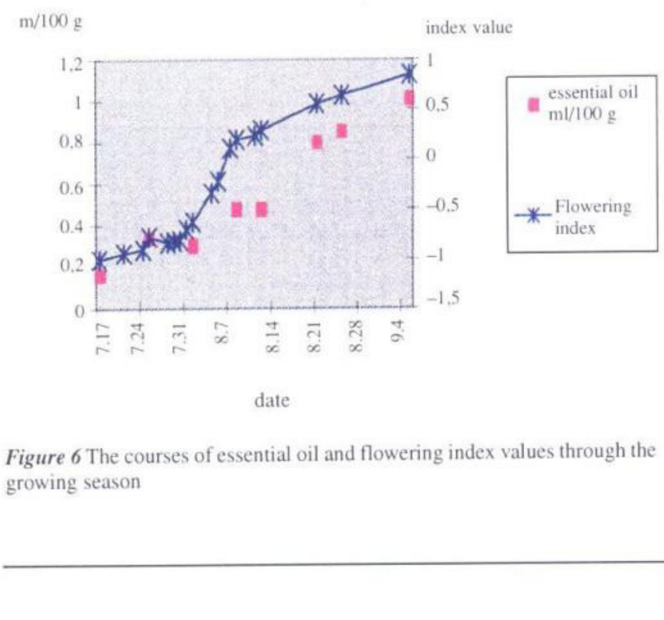


Figure 6 The courses of essential oil and flowering index values through the growing season

Table 2 Statistical parameters of the fitted sigmoid functions (non-linear regression analysis in SPSS)

	R2	parameters *	95% confidential interval	
			lower	upper
First individual I	0,98162	P1	-1.437	-0.910
		P2	0.693	0.892
		P3	0.186	0.360
		P4	13.075	15.840
Ia	0.99428	P1	-1.171	-0.974
		P2	0.780	0.961
		P3	0.268	0.428
		P4	25.382	26.683
Ib	0.97613	P1	-1.652	-0.822
		P2	0.661	1.059
		P3	0.114	0.391
		P4	22.164	26.443
II	0.98302	P1	-1.502	-0.877
		P2	0.574	0.876
		P3	0.163	0.439
		P4	22.466	25.674
V	0.98577	P1	-4.361	6.229
		P2	-4.873	6.741
		P3	-1.805	1.805
		P4	13.218	24.445

Table 2 cont.

	R2	parameters *	95% confidential interval	
			lower	upper
VI	0.95088	P1	-2.069	-0.500
		P2	0.424	0.949
		P3	0.036	0.564
		P4	19.853	26.925
average of the first individual	0.98810	P1	-1.052	-0.912
		P2	0.241	0.418
		P3	0.402	0.759
		P4	19.830	21.168
average of the first individual-main inflorescence	0.98412	P1	-1.090	-0.907
		P2	0.186	0.388
		P3	0.378	0.834
		P4	19.511	21.144
Second individual				
I	0.98562	P1	-1.611	-0.918
		P2	0.874	1.030
		P3	0.245	0.455
		P4	8.506	11.029
Ia	0.98329	P1	-1.155	-0.891
		P2	0.407	0.660
		P3	0.305	0.619
		P4	18.387	20.281
Ib	0.98944	P1	-1.201	-0.873
		P2	0.358	0.484
		P3	0.813	2.066
		P4	16.673	17.330
III	0.96444	P1	-6.019	1.754
		P2	0.685	1.438
		P3	-0.022	0.620
		P4	8.873	25.911
II	0.93819	P1	-25.139	17.195
		P2	0.280	0.871
		P3	-0.172	0.772
		P4	-14.065	38.333
X	0.98760	P1	-1.369	-0.822
		P2	0.323	0.617
		P3	0.267	1.038
		P4	20.934	22.772
XIII	0.97166	P1	-1.564	-0.671
		P2	0.218	0.523
		P3	0.177	1.118
		P4	17.622	21.010
average of the second individual	0.98964	P1	-1.075	-0.855
		P2	0.506	0.762
		P3	0.197	0.324
		P4	18.698	20.789
average of the second individual-main inflorescence	0.98831	P1	-1.173	-0.960
		P2	0.422	0.679
		P3	0.231	0.390
		P4	18.872	20.821
third individual				
I	0.99400	P1	0.943	1.057
		P2	-4.233	-0.953
		P3	-0.280	-0.161
		P4	-0.035	8.212
Ia	0.98665	P1	-1.232	-0.935
		P2	0.678	0.900
		P3	0.280	0.547
		P4	15.708	17.186
Ib	0.99479	P1	-1.076	-0.957
		P2	0.486	0.613
		P3	0.684	1.141
		P4	19.506	20.361
III	0.96684	P1	-1.187	-0.859
		P2	0.407	0.654
		P3	0.328	1.130
		P4	15.568	17.055

Table 2 cont.

	R2	parameters *	95% confidential interval	
			lower	upper
II	0.97751	P1	-1.231	-0.896
		P2	0.666	1.001
		P3	0.273	0.619
		P4	17.296	19.353
V	0.98189	P1	-1.381	-0.881
		P2	0.470	0.728
		P3	0.311	0.807
		P4	17.290	19.211
IX	0.92397	P1	-1.671	-0.632
		P2	0.102	1.217
		P3	-0.013	0.680
		P4	21.489	27.692
X	0.98874	P1	-1.107	-0.866
		P2	0.443	0.638
		P3	0.391	0.740
		P4	18.538	20.024
average of the third individual	0.95974	P1	-1.057	-0.786
		P2	0.527	0.902
		P3	0.159	0.459
		P4	24.421	27.619
average of the third individual–main inflorescence	0.98371	P1	-1.377	-0.912
		P2	0.572	0.874
		P3	0.151	0.386
		P4	24.181	27.064

*see equation in text

utilized also to define the phase when the essential oil attains its maximum level. Comparison of the essential oil accumulation and the flowering index data in a common system of co-ordinates is presented in *Fig. 6.* In the case of the studied population the essential oil reaches its maximum value at the end of flowering period which is characterized by 0.8-0.9 values of the flowering index. The two parameters show close correlation (**r**=0.964) at high probability. The definition of any phenological phase by the use of flowering index developed offers a new and objective method compared to those found in the earlier publications. It can be used also for the determination of optimal harvesting time.

By these results it was shown, that "full-flowering" as generally accepted definition of the phenological phase when the highest essential oil level and yield can be obtained, may be inadequate.

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