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Influence of Cold Temperature and Light on the Flower Induction of Thyme (*Thymus vulgaris* L.)

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Summary

Thyme (*Thymus vulgaris* L.) is a medicine and spice plant, which is cultivated in Poland, Spain, the South of France, Italy, Greece and Germany. The full flower of thyme plants can be induced only after winters with low temperatures. An artificial vernalisation at 3 °C for six weeks induced the flowers. Sixteen hours lighting were needed for an optimal development of the full flowers. The artificial flower induction can accelerate the generation cycle of thyme. Crossing works were independent of the natural development rhythm of plants. The seed yields of progeny individuals with vernalisation can be increased. Additionally, vernalisation increased the herb yields in autumn. The vernalisation can be used to accelerate breeding programs and to improve the appearance for sale as ornamental plant.

Key words. flower – induction – *Thymus vulgaris* – vernalisation

Introduction

The trend towards a healthier diet as well as the increasing recognition of the therapeutical potential of vegetable preparations forms the base for a growing demand for vegetable food. The use of vegetable raw material in the drug production is frequently more economical than the synthesis of chemical substances. The herbs thyme, oregano, basil and marjoram are medicine and spice plants. The need of Thymi herba (thyme herb) and of Thymi aetheroleum (thyme oil) led to an extensive thyme cultivation in Spain, France, Italy, Bulgaria and Eastern Europe. Only 170 ha of thyme are cultivated in Germany. The farmers must have more interesting varieties. Therefore breeding works are necessary. The improvement of the quality of the herbs and the increase of the essential oil content are aims for the breeders. A conventional method is the crossing of plants. Thyme induces the full flowers in the second vegetation year after a cold period in winter. A method of the artificial induction of flowering in the first year of cultivation could accelerate the breeding works. In addition, the induction of flowers of the crossing partners could be synchronized, because the flower induction of the hermaphroditic plants is mostly earlier than the flower induction of male sterile plants (Ehlers and Thompson 2004). A method for the flower induction is the artifical vernalisation.

Plant species in regions with pronounced temperature differences between winter and summer induce the flowers after a cold spell (Diomaiuto-Bonnand et al. 1980; Wiebe et al. 1992; Wiebe 1998; Duchovskiene und Bobinas 2000; Monaghan et al. 2004). Due to the reduced breeding works in Lamiaceae there were only few publications which described the flower induction by vernalisation. The flower formation was induced by vernalisation in *Lavandula angustifolia* (Monaghan et al. 2004). A work of Novak et al. 2005 described the vernalisation impact on the inducing of flowers in *Salvia officinalis*.

In this work we present the results of three single tests. The report gives information about the effects of vernalisation on the induction of flowers in thyme and the consequence for plant breeding.

Materials und Methods

Plant materials

The plant materials came from gene banks or from regular breeding programs. Coding and origin are listed in Table 1.

Conditions in the climatic chamber and in the greenhouse

The plants with growth heights higher than 10 cm were transferred into the cold chamber for six weeks. Prior to the experiments we found out that with a vernalisation period of less than six weeks the plants induced no flowers. Vernalisation conditions: temperature: 2.5–3.5 °C (target temperature 3 °C), light: 8 hours (6.00–14.00) and ca 2000 lux, air humidity: between 65 and 75 %. The control plants in the greenhouse were cultivated under constant conditions (temperature: 20–25 °C and daylight). At the end of vernalisation, the plants of experiment I were returned into the greenhouse. The vernalised plants of the experiments II and III were transferred together with the control plants into the experimental field.

Table 1. Plant materials of experiment I, II and III.

Experiment	Plants (n)	Number in the experiment	Cultivar	Institute
I	4 vegetatively propagated plants of each number	I-1	220 460 /'Skoneczko'	Gene bank, Radzikow, Poland
		I-2	56647	Gene bank, Braunschweig, Germany
		I-3	'Deutscher Winter'	Dr. Junghanns GmbH, Germany
		I-4	'Lithuania'	Horticulture Institute Kaunas, Lithuania
		I/5	THY 1/99	Gene bank Gatersleben, Germany
		I/6	'Varico II'	Delley Seeds and Plants AG, Switzerland
II	2 vegetatively propagated plants and 1 mother plant of each number	II-1, -2, -5, -6, -7, -14, -15, -17, -18	'Deutscher Winter'	Dr. Junghanns GmbH, Germany
		II-3, -4, -16	EPN, JUN 3	Dr. Junghanns GmbH, Germany
		II-11, -19	EPN, JUN 5	Dr. Junghanns GmbH, Germany
		II-10, -12, -13, -20	EPN, JUN 7/1	Dr. Junghanns GmbH, Germany
		II-8, -9	00-thy-110	C.N.P.M.A.I., France
III	20 plants of each number	III-1, -2, -3, -4	'Deutscher Winter'	Dr. Junghanns GmbH, Germany

Duration of the additional light in the greenhouse

At the end of vernalisation the plants were cultivated under additional lighting (Diomaiuto-Bonnand et al. 1980; Weiqi et al. 2002; Monaghan et al. 2004). A day lengths diagram for the location of Quedlinburg was provided (Moorberg) and the means of the day lengths from 11 May to 4 June were determined (Fig. 1). The reason why we chose this time period was the beginning to flowering of variety 'Deutscher Winter' in 2003 (Fig. 1). The variety 'Deutscher Winter' is frequently cultivated in Germany. The length of the necessary additional lighting in the winter was determined from the difference of the mean of the day lengths in May/June (16 h) and the day lengths during the winter months (8 h).

Statistic analysis

The statistic evaluation was accomplished with the procedure glm (means/Tukey LINES) of the statistic program SAS, version 8.0 for Windows.

The Experiments

Experiment I (Fig. 2)

Raw materials were four vegetatively propagated descendants of six different genotyps (Table 1). A method of the vegetative propagation of thyme is the propagation by cuttings with rooting hormones. Two of four descendants of each plant were subjected to cold temperatures for six weeks. At the same time the other two descendants remained under constant conditions in the greenhouse. After vernalisation one of two cold-treated plants and one of two untreated plants were cultivated in the greenhouse under supplementary light (descendant 1: vernalisation and additional light and descendant 2: without vernalisation and with additional light). The other two plants were cultivated under normal light conditions (descendant 3: vernalisation and normal light, descendant 4: = control plant without vernalisation and with normal light).

Evaluation: date of the initiation of buds (5 buds visible), date of the first flowers (5 flowers), date of full flower and flowering period (d).

Results. Only vegetatively propagated plants with vernalisation and additional lighting induced the flowers. The first flowers were opened in the time interval of 36 to 49 days after the end of vernalisation (Fig. 3). The other plants, without cold temperature and/or without additional light, remained non-flowering.

Experiment II (Fig. 2)

The aims of the experiment II were the induction of flowers and the generative reproduction of vegetatively propagated plants in the first year after propagation. Normally the production of seeds is impossible, because the progeny individuals do not flower in the first cultivation year.

In this experiment, two-year old hermaphroditic plants (mother plants, n=20) with different origins were vegetatively propagated in autumn (Table 1). Of each mother plant, two cuttings were produced. The mother plants stood in the experiment field in Quedlinburg. One rooted cutting of each mother plant was treated at 3 °C for six weeks. At the same time the other descendant was cultivated in the greenhouse with normal lighting and 20 °C. On 15 April 2004 the vegetatively propagated descendants were planted in an appropriate distance to the

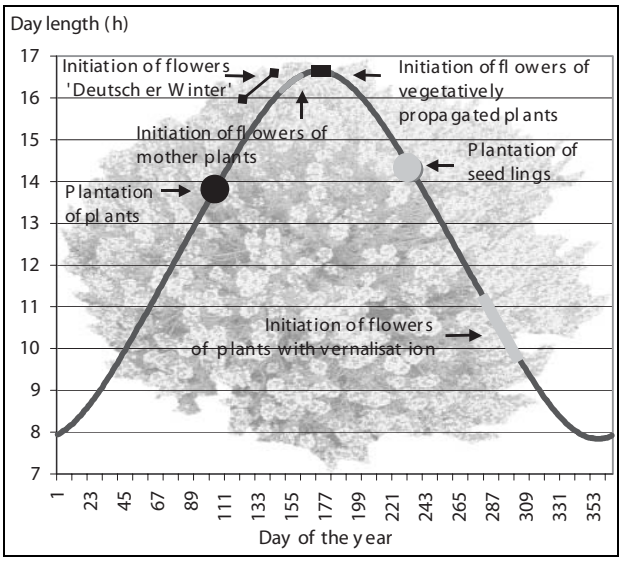


Fig. 1. A day length diagram for the location of Quedlinburg (Germany) and the most important times of the experiments II (black) and III (grey).

mother plants. The isolating caps preventing cross-pollination were put over the mother plant and the two vegetatively propagated descendants in May. The pollen grains were transferred via bees and with a brush. The bush volumes of plants were determined at full flowers in June/July (bush volume = maximum of growth height * maximum of bush diameter). In August the plants were harvested. The cut of the plants was 5 cm above the soil. All plants were air-dried. The dried thyme bush was cut and the seeds were collected. The thousand seed weight (g) and the germination ability (%) were determined in the laboratory.

Results. Two mother plants (numbers 16 and 19) could not be vegetatively propagated. The vernalised and the unvernalised progenies of the others showed no morphological differences on the day of plantation. In May, the mother plants began with flowering. All vegetatively propagated progeny individuals with vernalisation formed the flowers one month later. The flowering began in the time between 18 June and 29 June 2004, 5–7 weeks after the end of the vernalisation. Twelve of eighteen of the untreated progeny individuals were flowerless. The other six plants showed a reduced flower induction.

The mean of the seed yields of eighteen mother plants was 1.9 g. The mother plant 6 produced the highest seed yield (8.6 g), the mother plant 17 had the lowest seed yield (0.19 g, Table 2). The mean of the seed yields of seventeen vernalised progeny individuals was 0.5 g. The plant with number 20 was damaged. The vernalised progeny individuals of the mother plants 6 and 10 had the highest seed yields (>1 g per plant). The lowest seed yield had the progeny individual of the mother plant 15 (2 grains). The seed yields of six untreated progeny indi-

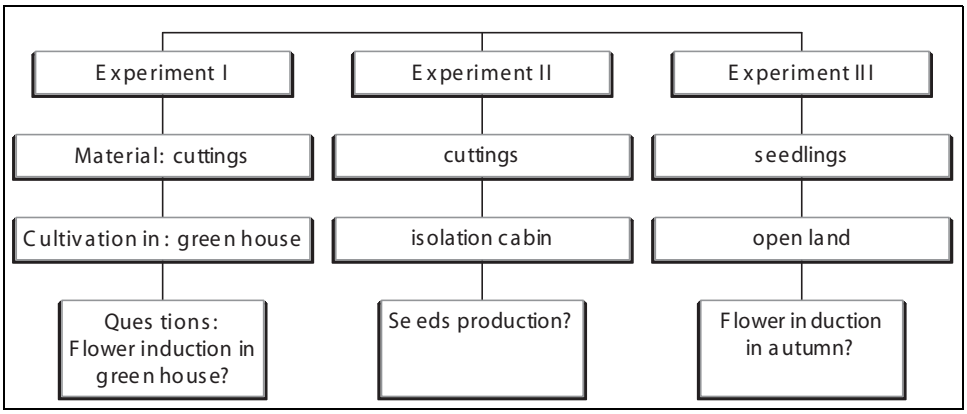


Fig. 2. Overview of the experiments with materials and questions.



Fig. 3. Left: Induction of flowers with cold temperature and light in green house. Middle: only cold temperature or only light. Right: without cold temperature and light.

viduals varied between 0.3 g and 30 grains. Correlations between the seed yields of mother plants and progeny individuals were not existent.

The reasons for the higher seed yields of mother plants were the volumes of mother plants, because bigger bushes formed more flowers, which can produce more seeds.

The means of the bush volumes of the mother plants varied between 46042 and 50086 cm^3 and the means of bush volumes of the progeny individuals between 4737 and 5189 cm^3. Significant differences in the thousand seed weight (mean = 3.16 g) and in the germination of seeds (mean = 95 %) between the mother plants and its progeny individuals were not available.

Experiment III (Fig. 2)

The influence of the vernalisation on seedlings of the cultivar 'Deutscher Winter' should be determined in a field experiment. The sowing was on 22 March 2004. All seedlings grew in the greenhouse under the same conditions. On 05 July 2004, some seedlings were vernalised, the other plants were cultivated in the greenhouse. At the end of the vernalisation time the seedlings were planted in the experimental field in four repetitions with á 20 plants (two repetitions vernalised plants, two repetitions non-vernalised plants).

The following traits were evaluated: the dates of the first flowers and the dates of full flowers, the growth heights (cm) and the maximums of bush diameters (cm). The bush volumes were calculated with the formula for the computation of a cone volume:

$V = (\pi * \text{maximum of bush diameter}^2 * \text{growth height}) / 3$ (Pank and Krüger 2003).

Results. On the day of plantation the vernalised plants and the unvernalised plants showed no morphological differences. Two and a half weeks later the cold treated plants showed a rise of the vegetative organogenesis. Altogether 38 plants with vernalisation and 27 plants without vernalisation could be evaluated. The initiations of the flowers of the plants with vernalisation were between 8 October and 30 October 2004. The full flowers were reached between 19 October and 8 November 2004, but the dimensions of the full flowers were smaller than the full flowers in the spring. The plants without vernalisation were flowerless. On the day of full flowers of the vernalised plants we determined the three features, growth height, maximum bush diameter and bush volume. We found out that the differences between the plants with and without the vernalisation were significant (Table 3). The growth heights of the plants with vernalisation varied between 13–23 cm, the growth heights of the plants without vernalisation were 3–12 cm. The bush diameters of the vernalised plants (15–31 cm) were greater than the bush diameters of the untreated plants (2–6 cm). The bush volumes, which were calculated from growth heights and maximum bush diameters, varied between 23411 and 3216 cm^3 (vernalised plants) and between 20 and 339 cm^3 (plants without vernalisation).

Table 2. Experiment II: seeds yield per plant (g)

Genotype	Seeds yield (g)		
	Mother plant	Progeny individuals with vernalisation	Progeny individuals without vernalisation
II-1	0.77	0.17	0.04
II-2	1.70	0.62	0.27
II-3	0.31	0.23	0.07
II-4	2.28	0.98	0.00
II-5	1.02	0.37	0.00
II-6	8.59	1.22	0.00
II-7	3.17	0.66	0.00
II-8	3.32	0.48	0.00
II-9	3.11	0.65	0.00
II-10	1.17	1.01	0.00
II-11	0.20	0.46	0.14
II-12	2.67	0.31	0.09
II-13	2.05	0.45	0.11
II-14	3.26	0.61	0.00
II-15	0.97	0.00	0.00
II-17	0.19	0.00	0.00
II-18	0.71	0.21	0.00
II-20	0.33	Damaged	0.00

Discussion

Our experiments showed that the vernalisation time of six weeks and 3 °C induced the flowers of thyme. The optimal development of the full flowers required a subse-

Table 3. Comparison of seedlings with and without vernalisation in autumn 2004.

Treatment	Number in the experiment	N	Height of plant (cm)		Maximum bush diameter (cm)		Volume of plant (cm^3)	
			Mean	Tukey	Mean	Tukey	Mean	Tukey
Plants with vernalisation	III-1, -3,	38	16,83	A	22,21	A	8037,84	A
Plants without vernalisation	III-2, -4,	27	6,59	B	3,63	B	91,63	B
GD (Tukey α = 5 %)			9,25		8,56		809,62	

quent day length of 16 h. The kind of the propagation of the plants (vegetative or generative), the age and the volume of the plants and the genetical constitutions had no influence on the results of the vernalisation. Only the vegetatively propagated individuals of vernalised mother plants did not induce the flowers (results are not presented).

A reason for the limited restricted flower formation in autumn could be the shorter day length (Fig. 1). In the months September and October the day lengths were 5-6 h shorter than in May and June. The limited day lengths influenced the flower induction. Full flowers were not induced. Devernalisation could be possible. Devernalisations were observed for example in *Apium graveolens* and *Brassica oleracea* (WIEBE et al.1992; WIEBE 1998).

Nevertheless, the vernalisation of seedlings increased the herb yields in the first cultivation year of thyme. Usually the yields of thyme are only higher after the winter in the second year (PANK and KRÜGER 2003). Vernalisation promoted also the vegetation in *Salvia officinalis* (NOVAK et al. 2005). The vernalisation time was the same as thyme, but the temperatures were 10-12 °C.

The use and the profitability of the vernalisation method in the commercial cultivation of thyme plants still have to be discussed. The advantages of the vernalisation can be seen in the breeding works. Crossing works are possible in the whole year in the greenhouse. In addition, flowering thyme plants could be provided with lower effort as ornamental plants. The flower colours varied from dark violet to white.

In the context of the crossing program at the BAZ in Quedlinburg and in the context of the examinations of the flower biology, the artificial flower induction was very useful (MEWES and PANK 2004).

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