The New *Thymus vulgaris* L. Hybrid Cultivar 'Varico 3' Compared to Five Established Cultivars from Germany, France and Switzerland

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Keywords: breeding, essential oil, homogeneity, thyme, yield

Abstract

In order to optimise quality and yield performance of thyme (*Thymus vulgaris* L.) a breeding programme was carried out and 56 new hybrids were obtained by crossing male sterile and male fertile clones. These hybrids were tested from 2000 to 2002 by evaluating homogeneity, yield in dry weight and essential oil, winter frost tolerance, as well as seed production potential of the parents. The most promising hybrid was named 'Varico 3' and was then compared to five established cultivars from Germany, Switzerland and France in mountain regions of Switzerland (920 m a.s.l.) in 2007 and 2008. The trial was harvested once in 2007 and twice in 2008. The two thymol-chemotype cultivars 'Varico 3' and 'Varico 2' showed the highest dry weight yields. 'Deutscher Winter', also a thymol-chemotype, had the lowest essential oil content with less than 3%. 'Varico 3' and 'Varico 2' contents were much higher with 4.9% and 3.5%, respectively. The highest essential oil contents were obtained with the three French cultivars, but with lower dry matter production. In general, the hybrid cultivars showed a higher homogeneity than the population cultivar 'Deutscher Winter'. In conclusion, the new hybrid cultivar 'Varico 3' can be recommended for the producers in Switzerland and middle Europe and for the industries looking for thymol-chemotype thyme.

INTRODUCTION

Thyme (*Thymus vulgaris* L.) is especially well known for its aromatic and therapeutic properties due to the essential oil of its leaves. The essential oil is used as a flavour additive, as well as an antimicrobial and antioxidative product (Mewes et al., 2008; Wichtl and Anton, 2003). According to the PhEur, an essential oil content of ≥1.2% v/w with at least 40% of thymol and carvacrol is required. In Switzerland, thyme is one of the main cultivated aromatic plants. It is mainly used by the food industry for the production of candies, herbal teas or ice tea.

The processing industry needs high quality, homogenous and standardised raw material. Thus it is important for thyme producers to have high quality cultivars with a good level of homogeneity. Rey (1993), Pank and Krüger (2003) and Rey et al. (2004) showed that crossing male sterile (MS) with male fertile (MF) plants to breed hybrids is an adequate approach to improve homogeneity of thyme cultivars. Therefore a breeding programme was conducted to optimise the quality, the yield performance and the homogeneity of thyme by exploiting the natural gynodioecy of thyme flowers (male sterile and hermaphroditic plants).

The aim of this study was to test the agronomic and therapeutic properties of the 56 new thyme hybrids (thymol-types). The most appropriate hybrid was then evaluated in comparison to commercial thyme cultivars from Germany, France and Switzerland.

MATERIALS AND METHODS

Breeding programme: 17 thyme accessions (12 MS and 5 MF) were collected from three sources: a) wild populations in the Aosta Valley (Northern Italy, n=6 MS and n=3 MF), b) breeding material from Agroscope ACW (n=4 MS and n=2 MF) and c) 'Deutscher Winter' commercial population-cultivars (n=2 MS). These accessions were vegetatively propagated and then crossed. A total of 56 new hybrids were obtained by

crossing male sterile and male fertile clones. The hybrids were then tested for cultivation during three years in mountain regions of Switzerland in Arbaz (VS, altitude 920 m, southern exposure; 20% slope, sandy soil, pH 7.8). Seeds were sown in greenhouse in March 2000 and then transplanted in seedtrays 4 weeks later. In May 2000, the seedlings were planted in the field at Arbaz (5.7 plants/m^2; 0.7 m × 0.25 m per plant). Per plot 25 plants on one row (6.25 m) were considered. Five harvests were carried out: 4.9.00, 5.6.01, 11.9.01, 18.6.02, 24.9.2002. Hybrids were evaluated by analysing, yield in dry weight and essential oil, as well as the seed yield by crossing the respective parents (12 plants of MS and 6 plants of MF cultivated in pots).

Cultivar comparison: six cultivars (Table 1) were tested in Arbaz. Seeds were sown in greenhouse in March 2007 and then transplanted in seedtrays 4 weeks later. In May 2007, the seedlings were planted in the field at Arbaz (9.8 plants/m^2). Per plot 54 plants grown in three rows on a bed of (1.4 m × 4 m) were considered. Three harvests were carried out up to now: 3.9.07, 3.6.08, 10.9.08. The cultivars were evaluated for their homogeneity by collecting 10 stems per replication according to a defined scheme. Different plant traits were then measured such as length of stems, distance between internodes from the 5^{th} to the 6^{th} leaf and leaf length of leaves from the 5^{th} internode. Yield in dry weight and essential oil, as well as winter frost tolerance were also considered.

The experimental design consisted of a complete randomised block with 4 replications. The fertilisers, authorized in organic farming (Biorga NR, Granuphos, Patentkali), were used according to the guidelines of fertilisation for thyme (in kg per hectare: 60 N, 30 P_2O_5, 100 K_2O and 15 Mg) (Carlen et al., 2006). The nitrogen application was done twice (one in spring and one after the 1^{st} harvest). Thyme fields were irrigated when necessary. No plant protection treatment was applied. The weeding was done manually between the plants and with a harrow between the lines. Cutting height was 5 cm above the soil surface. The harvested plants were dried at a temperature of 35°C.

Hydro-distillation was done according to the PhEur. Oil samples were stored at 4°C. The composition of essential oil in the leaves was quantified with gas chromatography (GC/FID) by the laboratory ILIS Sàrl, Bienne, Switzerland.

RESULTS AND DISCUSSION

The breeding programme yielded 56 new hybrids obtained by crossing male sterile and male fertile clones. Some of them showed very promising results (Table 2). In particular, the hybrid 3 (‘Varico 3’) obtained by crossing two accessions from the Agroscope ACW breeding material showed a high essential oil content with 4.4% average over 5 harvests and a high leaf yield. In addition, the parents of this hybrid are well synchronised in their flowering period and have quite a good seed production potential. This new hybrid cultivar was named ‘Varico 3’. The comparison of 56 hybrids showed that thyme hybrids with a high biomass production are not necessarily characterised by low content of essential oil (Fig. 1). This indicates that processes underlying dry weight production and oil production are not strongly related. This gives the opportunity to breed for increasing yields and essential oil content concomitantly.

The new hybrid cultivar ‘Varico 3’ was then compared to other cultivars from Germany, Switzerland and France to evaluate its behaviour in mountain regions (Table 1). The thymol-chemotypes cultivars ‘Varico 3’ and ‘Varico 2’ showed the highest dry weight yields (Tables 3, 4). ‘Deutscher Winter’ (thymol-chemotype) and the hybrid cultivar ‘122’, a thymol-carvacrol type, produced about 25% less dry weight yield. The lowest yields in mountain regions were obtained with the two French cultivars ‘L2’, a linalool-type, and ‘147’, a carvacrol type. The highest leaf-flower/stem ratio was observed with ‘Deutscher Winter’ and ‘L2’. In contrast, ‘Deutscher Winter’ had the lowest content in essential oil (less than 3%). ‘Varico 3’ had about 75% and ‘Varico 2’ 25% higher contents than ‘Deutscher Winter’. The highest essential oil contents were obtained with the three French cultivars. No winter frost damages were registered for all

the cultivars.

The homogeneity of thyme strongly depends on the cultivar. The lowest average coefficient of variation (CV) was observed with 'Varico 3', based on plant height, internode length and leaf length. The population-cultivar 'Deutscher Winter' had a high CV for all the traits analysed. This confirms the usefulness of hybrid breeding for the improvement of homogeneity (Pank and Krüger, 2003).

CONCLUSIONS

The new hybrid cultivar 'Varico 3' can be recommended for the producers in Switzerland and middle Europe and for the industries looking for thymol-chemotype thymes. 'Varico 3' shows a high homogeneity, a good dry weight yield performance and a high essential oil content in the leaves.

ACKNOWLEDGEMENTS

The authors thank the Swiss Federal Office for Agriculture and Ricola AG for their financial support, as well as Charly Rey (Agroscope ACW), Ivan Slacanin (ILIS Sàrl, Bienne), Bénédicte Bruttin (Agroscope ACW) and Sarah Bouillant (mediSeeds) for their assistance.

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Tables

Table 1. Characteristics of the six cultivars evaluated in Arbaz in 2007 and 2008.

Cultivar	Category	Breeder	Commercial Company
'Varico 3'	Hybrid	Agroscope ACW[a], CH	mediSeeds[b], CH
'Varico 2'	Hybrid	Agroscope ACW, CH	mediSeeds, CH
'Deutscher Winter'	Population cultivar	-	UFA[c], CH
'L2'	Hybrid	Iteipmai[d], F	Iteipmai, F
'112'	Hybrid	Iteipmai, F	Iteipmai, F
'147'	Hybrid	Iteipmai, F	Iteipmai, F

[a] Agroscope Changins-Wädenswil Research Station ACW, Research Centre Conthey, 1964 Conthey, Switzerland
[b] mediSeeds sàrl, Route des Vergers 18, 1964 Conthey, Switzerland
[c] Fenaco, UFA-Samen, Obstfeldstrasse 1, 6210 Sursee, Switzerland
[d] Iteipmai, Institut technique interprofessionnel des plantes à parfum, médicinales et aromatiques, BP 09 Melay, 49120 Chemillé, France

Table 2. Comparison of 56 new hybrids of thyme. Yields and leaf essential oil contents correspond to the sum/mean of 5 harvests (2000-2002) in Arbaz (4 replications). The seed yield was obtained in a pot experiment (no replications). 'Varico 3' is highlighted in bold.

Accessions crossed[a] MS × MF	Hybrid number	Total dry mass yield (t/ha)	Leaf dry mass yield (t/ha)	Essential oil content (% v/w)	Essential oil yield (L/ha)	Seed yield (g/plant)
DW-1 × ACW-b	48	13.1	7.3	3.6	261	0.3
ACW-1 × ACW-a	**3 ('Varico 3')**	**12.6**	**6.5**	**4.4**	**288**	**0.4**
DW-1 × A-c	38	12.3	6.7	4.5	300	0.1
A-2 × ACW-b	46	11.5	6.0	3.8	229	0.2
A-1 × ACW-b	45	11.5	6.5	4.3	278	0.4
DW-2 × ACW-b	49	11.4	6.4	3.5	221	0.1
DW-1 × A-a	16	11.4	6.1	3.8	230	0.5
A-2 × ACW-a	2	11.2	6.0	4.3	258	0.2
A-4 × ACW-b	52	11.1	6.1	3.7	230	0.7
DW-1 × ACW-a	4	11.0	6.1	3.7	225	1.0
A-4 × A-c	42	10.8	5.9	4.3	255	0.1
A-1 × ACW-a	1	10.3	5.8	4.6	266	0.1
A-6 × ACW-a	12	9.8	5.4	4.9	268	0.2
A-5 × ACW-a	9	9.8	5.5	4.5	247	0.4
ACW-2 × ACW-b	50	9.8	5.5	3.7	203	0.6
A-4 × ACW-a	8	9.7	5.2	3.9	204	0.4
A-5 × ACW-b	53	9.7	5.5	4.5	247	0.3
A-3 × ACW-a	7	9.7	5.4	4.4	237	0.7
A-4 × A-b	31	9.5	5.2	4.4	227	1.0
DW-1 × A-b	27	9.5	5.2	4.0	209	0.2
A-3 × ACW-b	51	9.2	5.1	4.1	212	0.4
ACW-3 × ACW-a	10	9.0	5.0	3.8	189	0.8
ACW-2 × A-c	40	8.9	4.9	4.2	204	0.2
ACW-3 × ACW-b	54	8.8	5.0	4.1	206	0.4
ACW-2 × A-a	18	8.8	4.9	3.7	184	1.0
ACW-2 × A-b	29	8.7	5.0	4.3	217	1.0
A-6 × ACW-b	56	8.6	5.0	4.6	229	0.1
A-2 × A-c	36	8.6	4.6	4.5	208	0.1
A-4 × A-a	20	8.5	4.7	3.7	174	0.6
ACW-4 × A-c	44	8.5	4.8	4.2	201	1.4
ACW-1 × ACW-b	46	8.4	5.0	4.1	201	0.8
ACW-2 × ACW-a	6	8.3	4.7	3.9	184	0.7
A-2 × A-b	25	8.0	4.3	4.2	177	0.4
DW-2 × ACW-a	5	7.8	4.6	3.5	160	0.5
ACW-1 × A-b	26	7.7	4.1	4.8	196	0.4
ACW-4 × ACW-a	11	7.7	4.2	4.0	166	2.9
ACW-4 × ACW-b	55	7.6	4.4	3.8	165	1.2
DW-2 × A-a	17	7.5	4.3	3.4	145	0.1
A-2 × A-a	14	7.5	4.1	4.1	168	0.1

Table 2. Continued.

Accessions crossed[a] MS × MF	Hybrid number	Total dry mass yield (t/ha)	Leaf dry mass yield (t/ha)	Essential oil content (% v/w)	Essential oil yield (L/ha)	Seed yield (g/plant)
ACW-3 × A-c	43	7.4	4.1	4.0	163	0.3
ACW-1 × A-a	15	7.2	4.1	4.3	178	0.4
ACW-1 × A-c	37	7.0	3.9	5.2	201	0.1
ACW-3 × A-a	22	6.9	3.8	4.0	154	0.5
ACW-4 × A-a	23	6.8	3.8	4.1	155	2.3
A-1 × A-b	24	6.3	3.5	5.1	178	0.5
ACW-4 × A-b	34	5.8	3.3	4.0	132	1.8
A-1 × A-c	35	5.8	3.3	4.8	156	0.6
A-5 × A-b	32	5.7	3.3	4.6	153	0.3
DW-2 × A-c	39	5.4	3.1	3.6	109	0.1
ACW-3 × A-b	33	5.4	3.0	4.3	127	0.6
A-1 × A-a	13	5.3	3.1	4.5	143	0.9
A-5 × A-a	21	5.1	2.9	4.1	119	0.3
A-3 × A-b	30	5.0	2.7	4.6	126	0.1
DW-2 × A-b	28	4.4	2.5	3.7	91	0.1
A-3 × A-a	19	3.1	1.7	3.9	66	0.1
A-3 × A-c	41	3.0	1.7	4.1	69	0.1
Fisher test (LSD; P< 0.05)		2.1	1.3	0.6	6.5	-

[a]MS=male sterile, MF=male fertile; assessions from ACW=Agroscope ACW, DW='Deutscher Winter', A=Aosta Valley

Table 3. Comparison of six cultivars of thyme. Yields and essential oil contents correspond to the sum/mean of 3 harvests (2007-2008) in Arbaz with 4 replications.

Cultivar	Total dw yield (t/ha) 07-08	Leaf proportion (%) $\bar{x}$ 07-08	Leaf dw yield (t/ha) 07-08	Essential oil content of leaves (% v/w) $\bar{x}$ 07-08	Essential oil yield (L/ha) 07-08
'Varico 3'	6.16 ab	63 b	3.86 ab	4.9 b	191 a
'Varico 2'	6.63 a	63 b	4.17 a	3.5 c	146 b
'Deutscher Winter'	4.91 c	67 a	3.31 c	2.9 d	97 c
'L2'	3.98 d	67 a	2.66 d	4.9 b	130 bc
'112'	5.37 bc	64 ab	3.43 bc	6.2 a	212 a
'147'	4.51 cd	66 ab	2.97 cd	5.1 b	150 b

Different letters indicate significant differences between the cultivars (Tukey-Test, P<0.05).

Table 4. Composition of the essential oil of six thyme cultivars at the 1st harvest in June 2008 in Arbaz.

Cultivar	Thymol (% v/v)	Carvacrol (% v/v)	Linalool (% v/v)	γ-Terpinene (% v/v)	p-Cymene (% v/v)
'Varico 3'	65	3	2	13	7
'Varico 2'	57	3	2	15	8
'Deutscher Winter'	56	3	2	16	9
'L2'	11	23	48	5	3
'112'	30	31	5	15	7
'147'	22	41	6	11	8

Table 5. Comparison of different plant traits between the six cultivars at the 2nd harvest in September 2008 in Arbaz. The values correspond to the mean of 12 measurements per replication and 4 replications. The coefficient of variation (CV) was calculated from 40 measurements per cultivar.

Cultivar	Stem length (mm)	(CV) (%)	length of internode (mm)	(CV) (%)	Leaf length (mm)	(CV) (%)
'Varico 3'	157 a	(17)	8.0 a	(18)	5.8 ab	(11)
'Varico 2'	148 a	(14)	7.3 ab	(23)	6.2 a	(13)
'Deutscher Winter'	141 ab	(21)	7.6 ab	(43)	5.5 ab	(18)
'L2'	124 b	(17)	5.7 b	(18)	5.2 b	(8)
'112'	153 a	(16)	6.8 ab	(27)	5.2 b	(13)
'147'	149 a	(19)	6.6 ab	(18)	5.8 ab	(11)

Different letters indicate significant differences between the cultivars (Tukey-Test, $P<0.05$)

Figures

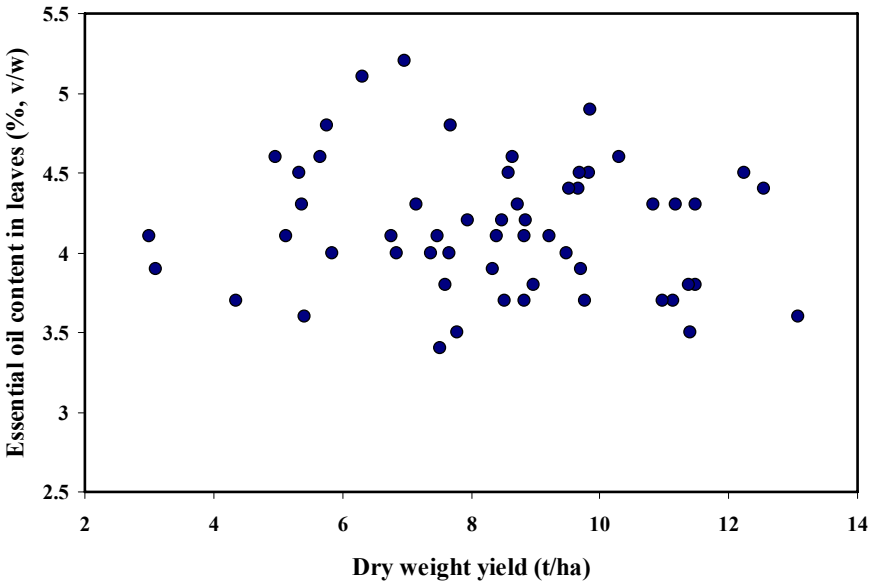

Fig. 1. Relationship between dry matter production and essential oil content of thyme leaves. Each point represents a hybrid (mean of 4 replications).