Agroscope launches 'Carola', a new variety of sage (*Salvia officinalis* L.)

X. Simonnet[1], B. Christ[1], C.A. Carron[1], J. Vouillamoz[1], M. Pramsohler[2], C. Carlen[1]

[1]Agroscope, Centre de recherche Conthey, Route des Eterpys 18, 1964 Conthey, Switzerland ; [2] Laimburg Research Centre, Laimburg 6 Pfatten (Vadena), 39040 Auer (Ora), BZ, Italy

Abstract

Common sage (*Salvia officinalis* L.) is one of the main species of aromatic plants cultivated in Switzerland. The almost total absence of characterized commercial varieties, but also the need to have an easy to multiply, high-performance variety adapted to cultivation in mountain areas, led us to set up a new breeding program for *S. officinalis*. Started in 2010, this work led to the creation of a new variety derived from a polycross and named 'Carola'. 'Carola' has been evaluated since 2020 at 2 locations in Switzerland, in comparison with the former variety 'Regula' and the German variety 'Extrakta'. In the first year of cultivation (1-2 cut), dry yields for this new variety ranged from 2.25 to 2.75 t ha^{-1} with no significant difference from the other 2 varieties. In the second year (2 to 3 cuts), 'Carola' is equivalent to 'Extrakta' with an average dry yield of 4.10 t ha^{-1}, and slightly higher than 'Regula'. The essential oil content of the leaves of this new variety, with 1.9 to 2.5% at the end of summer, is intermediate to the two control varieties, 'Extrakta' being the lowest.

The seeds of this new Agroscope variety 'Carola' will be available from the beginning of 2023 at mediseeds Sarl (www.mediseeds.ch).

Keywords: *Salvia officinalis*, aromatic plant, essential oil, breeding, polycross, 'Carola'

INTRODUCTION

Sage (also known as common sage or dalmatian sage, *Salvia officinalis* L.) has been the constituent of numerous traditional remedies. Nowadays, sage is mainly used as culinary herb, in beverages, as herbal product in the form of herbal teas, or extracts, in cosmetics and perfumery. Common sage is one of the most widely cultivated aromatic plants in Switzerland. Faced with the absence of a commercial variety and in order to meet the requirements of organic cultivation under Swiss climatic conditions, a first breeding program conducted in the 1990s led to the creation of a productive variety called 'Regula' (Carron et al., 2005). However, difficulties in producing seed for this clones hybrid variety led to the initiation of a new breeding program from 2010. In order to overcome the previous problem of low yields of commercial seed, but also to reduce the costs of seed production, the choice was made to create a polycross variety. Twenty accessions from various suppliers in Europe served as the genetic basis for this new program. Evaluated on agronomic (dry leaf yield, vigour) and phytochemical (essential oil content and composition) criteria, 10 hermaphroditic genotypes were pre-selected. In addition to their agronomic performance, these genotypes were specifically evaluated for their suitability for seed production. In the end, 4 genotypes from 4 different accessions were selected as parents for a polycross. Named 'Carola', the new resulting variety was evaluated at two Swiss sites in comparison with the former variety 'Regula' and the German variety 'Extrakta' during two consecutive years (2020-2021). This paper reports the results of these evaluations.

MATERIALS AND METHODS

The seedlings of the 3 varieties ('Carola', 'Regula', 'Extrakta') were produced by the company Biojardins (Fully, Switzerland).

The evaluations were carried out in Switzerland under real conditions on two fields in commercial production according to the Swiss Organic Farming rules (table 1).

The planting was done on 16 (site 2) and 25 May 2020 (site1) in beds of 3 lines at a density of 6.3 plants/m^2, with a minimum of 5 beds (550 m^2) per variety for each of the 2 sites. Depending on the site and the year, 1 to 3 cuts were made per year (table 1). The harvesting was carried out with a SuperCut trimmer on 2 m^2 plots repeated 4 times. After drying at 35-40°C, the stems were removed before analysis.
The determination of the essential oil content (v/w) was carried out on 20 g samples for 2 h using a hydro-distillation Clevenger apparatus system. The oil composition of the samples in the 2nd year (2021) was determined by GC-MS according to the NF ISO 11024 standard by the laboratory Pyrenessences Analyses (France).
The statistical analyses (ANOVA) were done according to block design by computer statistical program, XLSTAT. The differences among the means were compared using Tukey test.

Table 1. Sites location and harvesting times

Site	Location	Altitude	Year 2020	Year 2021
Site 1	Bannwil (BE) (47.23679, 7.73780)	415 m asl	1st cut: 18 August 2nd cut: 8 October	1st cut: 26 May 2nd cut: 9 July 3rd cut: 30 Sept.
Site 2	Sembrancher (VS) (46.07551, 7.16357)	730 m asl	1st cut: 25 August	1st cut: 17 June 2nd cut: 7 Sept.

RESULTS AND DISCUSSION

Biomass yields

The dates of the first cuts were made before flowering to meet the requirements of the Swiss market, which requires to minimize flowering stems in the harvest. Thus the milder climate of site 1 (Swiss plateau) favored earlier harvest dates and additional cutting in the 1st and 2nd year, compared to site 2 with a more alpine climate (table 1).
In the first yearthe average dry biomass yields of the 1st cuts were not significantly different ($p>0.05$) between the two sites, with 2.50 t ha^{-1} dry herb for site 1 and 2.58 t ha^{-1} for site 2 (table 2). However, a second cut at a fairly late stage of the season resulted in a 35% increase in productivity (0.9 t ha^{-1} dry herb) for site 1 compared to site 2. The new 'Carola' variety was penalized on the first cut (-15%) by a slower development after planting than for the other two varieties. But this difference was compensated with the second cut. Together, the two cuts of the 'Carola' variety at site 1 achieves a dry biomass yield equivalent to that of 'Extrakta'.
In the second year of cultivation, the annual dry herb yield reached an average of 3.83 t ha^{-1} (site 1) and 3.97 t ha^{-1} (site 2) with no significant difference ($p>0.05$) despite an additional cut on site 1 (table 3). The lower productivity of the 'Regula' variety on the first cut, on both sites, could most certainly be explained by a greater sensitivity to winter cold. This decrease in productivity of the 'Regula' variety was confirmed on the second cut of site 2, contrary to site 1 where the 3 varieties reached equivalent yields for the following cuts.
The yields obtained at these two Swiss sites are consistent with those obtained from trials conducted in Italy with the varieties 'Regula' and 'Extrakta' (De Maestro et al., 2006) and in Switzerland with the variety 'Regula' (Carlen et al., 2009).

Essential oil contents and yields

In the first year of cultivation, the behaviour of the varieties differs depending on the cultivation site. While for site 1, the three varieties have an equivalent essential oil content, on site 2, 'Carola' and 'Regula' differ from 'Extrakta' by a higher essential oil content (table 2). The same difference can be seen in the 2nd cut at the end of summer at site 1.

In the second year of cultivation, 'Regula' shows systematically higher contents than the other two varieties; this superiority being more pronounced at site 2.
The variety 'Carola', with contents similar to those of 'Extrakta' on the 1st cut (spring), is then at an intermediate level between Regula and Extrakta on the 2nd and 3rd cuts.

The differences in content observed between the two sites on the 1st cut in the 1st year (summer) ($p>0.05$) and 2nd year (spring) ($p>0.05$), are consistent with multi-site observations reported by other authors (Perry et al., 1999; Carron et al., 2005; De Maestro et al., 2006) Low essential oil contents in spring and then increasing in summer (Table 2) are also well known for this species (Anonymous, 1993; Carron et al., 2005). With the exception of the 1st cut at site 1 in the 2nd year, all other harvests were above the minimum essential oil content requirement (1.2% v/m) of the European Pharmacopoeia (9th ed.).

Although these trials were conducted with cut management for the production of dry plants and not for essential oil, the results nevertheless give a good indication of the production potential of the varieties tested. Thus, the yields of essential oil accumulated over the two years of evaluation are very similar between the varieties 'Carola' and 'Regula', with 95 l ha^{-1} and 102 l ha^{-1} respectively on site 1 and 77 l ha^{-1} and 75 l ha^{-1} on site 2. 'Carola' shows a productivity gain of 10 to 15% compared to 'Extrakta' (85 l ha^{-1}, site 1 and 70 l ha^{-1}, site 2).

Essential oil composition

The essential oil of *Salvia officinalis* is characterised by the presence of monoterpene ketones (α-thujone, β-thujone, camphor) and other oxygenated terpenes such as 1,8 cineole. These compounds show a very high variability within the *S. officinalis* species, but also according to the organs, stages and dates of harvesting and cultivation sites (Perry et al., 1999; Bagdat et al., 2017; Nguyen et al., 2019). The three varieties tested showed very little difference in composition between them, except for the 1,8-cineole on average lower in 'Regula' (Table 4). The higher camphor content in the first cut at site 2 is explained by a harvest done 3 weeks later than at site 1. Camphor content is indeed known to be lowest in spring before rising rapidly during summer (Carron et al., 2005). The accumulation dynamics of α-thujone, the major compound, observed during the season is also typical of this species, with a 2-fold increase (16 to 32%) between essential oils harvested in spring and those obtained at the end of summer.

Table 2. Biomass yield, essential oil (EO) content and yield during the first growing season (2020)

Varieties	**Dry herb yield (t ha^{-1})**		**Dry leaves (t ha^{-1})**		**EO content (% v/w)**		**EO yield (l ha^{-1})**	
	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
				1st harvest				
'Carola'	2.25 b	2.32	1.68	1.60	1.88	1.68 a	31.6	26.7
'Regula'	2.61 a	2.73	1.85	1.67	1.86	1.76 a	34.3	29.4
'Extrakta'	2.64 a	2.71	1.84	1.78	1.85	1.38 b	33.9	24.5
mean	**2.50**	**2.59**	**1.79**	**1.68**	**1.86**	**1.61**	**33.3**	**26.9**
				2nd harvest				
'Carola'	1.00	-	0.82 a	-	1.93	-	15.7 a	-
'Regula'	1.20	-	0.90 a	-	2.01	-	17.8 a	-
'Extrakta'	0.74	-	0.63 b	-	1.65	-	10.3 b	-
mean	**0.98**		**0.78**		**1.86**		**14.6**	
				Total				
'Carola'	3.25 b	-	2.50	-	-	-	47.3	-
'Regula'	3.81 a	-	2.75	-	-	-	52.1	-
'Extrakta'	3.38 b	-	2.47	-	-	-	44.2	-
mean	**3.48**		**2.57**				**47.9**	

Different letters indicate significant differences at $p<0.05$ (Tukey test)

Table 3. Biomass yield, essential oil (EO) content and yield during the second growing season (2021)

Varieties	Dry herb yield (t ha^{-1})		Dry leaves (t ha^{-1})		EO content (% v/w)		EO yield (l ha^{-1})	
	Site 1	**Site 2**	**Site 1**	**Site 2**	**Site 1**	**Site 2**	**Site 1**	**Site 2**
				1st harvest				
'Carola'	1.06	1.57 a	0.70 ab	0.90 a	0.89	1.51 b	6.1	13.9
'Regula'	0.73	0.91 b	0.51 b	0.54 b	1.18	1.91 a	6.0	10.6
'Extrakta'	1.12	1.33 ab	0.78 a	0.81 a	1.03	1.49 b	8.0	12.4
mean	**0.97**	**1.27**	**0.66**	**0.75**	**1.03**	**1.64**	**6.7**	**12.3**
				2nd harvest				
'Carola'	1.40	2.58 ab	0.87	1.80	1.95	2.01 b	16.9	36.1
'Regula'	1.47	2.39 b	0.95	1.59	2.05	2.24 a	19.4	35.4
'Extrakta'	1.37	3.13 a	0.84	1.98	1.74	1.66 c	14.5	33.0
mean	**1.41**	**2.70**	**0.89**	**1.79**	**1.91**	**1.97**	**16.9**	**34.8**
				3rd harvest				
'Carola'	1.61	-	1.16	-	2.14 ab	-	24.7	-
'Regula'	1.40	-	1.03	-	2.39 a	-	24.8	-
'Extrakta'	1.33	-	0.98	-	1.84 b	-	17.9	-
mean	**1.45**		**1.06**		**2.12**		**22.5**	
				Total				
'Carola'	4.07	4.15 ab	2.73	2.70 ab	-	-	47.7	50.0
'Regula'	3.60	3.30 b	2.49	2.13 b	-	-	50.2	46.0
'Extrakta'	3.82	4.46 a	2.60	2.79 a	-	-	40.4	45.4
mean	**3.83**	**3.97**	**2.61**	**2.54**			**46.1**	**47.1**

Different letters indicate significant differences at $p<0.05$ (Tukey test)

Table 4. Essential oil composition (major compounds) during the second growing season (2021); average values of the 4 repetitions

Varieties	**α-pinene** (%)		**1,8-cineole** (%)		**α-thujone** (%)		**β-thujone** (%)		**Camphor** (%)	
	Site 1	**Site 2**	**Site 1**	**Site 2**	**Site 1**	**Site 2**	**Site 1**	**Site 2**	**Site 1**	**Site 2**
					1st harvest					
'Carola'	5.98	8.27	14.15	11.74	17.54	16.39	7.24	6.43	1.97	11.34
'Regula'	6.01	3.89	16.28	13.28	14.86	14.93	7.07	7.15	1.37	12.36
'Extrakta'	4.73	5.89	14.89	12.86	16.05	16.00	7.35	6.92	1.63	8.94
					2nd harvest					
'Carola'	8.96	7.53	7.70	7.83	21.85	25.79	7.01	5.74	17.16	15.88
'Regula'	2.12	1.58	9.67	9.34	19.33	25.27	8.21	7.33	17.41	15.57
'Extrakta'	5.66	4.19	8.74	9.54	18.09	21.75	8.73	7.62	16.64	15.04
					3rd harvest					
'Carola'	6.27	-	7.19	-	32.80	-	5.48	-	15.53	-
'Regula'	1.50	-	8.16	-	31.07	-	6.57	-	16.54	-
'Extrakta'	5.84	-	7.34	-	33.04	-	5.54	-	14.65	-

CONCLUSION

The evaluation of the new Agroscope variety 'Carola', in comparison with the old variety 'Regula' and the German variety 'Extrakta', was successfully conducted in Switzerland at 2 sites with different soil and climatic characteristics.

With a dry biomass yield over 2 years of 6.5 to 7.3 t h-1, the variety 'Carola' reaches a production potential equivalent to that of the old varieties 'Regula' and 'Extrakta'. The essential oil content of 'Carola' is intermediate to that of 'Regula' and 'Extrakta', with a similar phytochemical profile.

The variety 'Carola', whose seeds are marketed by mediseeds Sarl (www.mediseeds.ch), can be recommended to Swiss producers as a replacement for the old variety 'Regula', which is no longer commercialized.

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