Industrial Crops & Products 170 (2021) 113764

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

In the shade – Screening of medicinal and aromatic plants for temperate zone agroforestry cultivation

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ARTICLE INFO

Keywords:
Biomolecules
Regenerative agriculture
Medicinal agroforestry

ABSTRACT

Agroforestry is one of the best land use management systems that regenerate degraded agroecosystems while maintaining high productivity. However, current knowledge about how shade from trees affects the cultivation of medicinal and aromatic plants in temperate zones is lacking. Therefore, the authors explored the effects of shade on the most important cultivation parameters of medicinal and aromatic plants. A three-year-long open field study was conducted with a control (C) and two artificial shade treatments (30 % - T1 and 50 % - T2 light intensity decrease). Shade effects on seven different species were evaluated for plant height and width, fresh yield, drug mass, essential oil content, and content of other biologically active compounds. Our first hypothesis was confirmed, because we observed pronounced species-specific shade tolerance. Secondly, it was proven that there are species of medicinal and aromatic plants — grown in temperate climates — for which it is possible to produce the quality of drug specified in the professional standards. They provide adequate yields even under mild (30 %) shade conditions. Consequently *Calendula officinalis* L., *Dracocephalum moldavica* L., *Melissa officinalis* L. and *Satureja hortensis* L. are highly recommended for further agroforestry experiments in large-scale and authentic agroforestry conditions. Mild shade (30 %) had favourable effects on several species in our experiments; however, 50 % shade produced no favourable effect on any examined species. Therefore, we suggest temperate zone medicinal agroforestry systems be designed such that the shadow should not exceed 30 %.

1. Introduction

Agricultural and horticultural production has attained the highest yields which cannot be increased without further negative externalities or rising expense. The ecological fragility of the uniform, extreme monocultures has been demonstrated repeatedly over the last centuries (Townsend et al., 2008). The Fourth Agricultural Revolution is characterized both by the appearance of digital and circular systems in agricultural management; and immersion in traditions and patterns of nature (Lombardo et al., 2017; Toop et al., 2017; Stojanovic, 2019; Gyuricza and Borovics, 2018). The future of land use is facing many pressing challenges, so the imperative of sustainable agriculture needs to expand to regenerative agriculture for the benefit of the next generations (Soloviev, 2014; Rhodes, 2017). Agroforestry systems (AFS) are the reorganizations of an old-but-new concept where agriculture and forestry are combined on the same land (Nerlich et al., 2013; Mosquera-Losada et al., 2018). AFS are subsidized more and more, thus they may be one of the flagships of the regeneration process owing to their advantageous effects. For example: maintaining ecosystem-services and biodiversity, sequestering carbon, conservation of soil, preserving landscape and cultural heritage, supporting soil food webs, pollination and biological control, and improving competitiveness of farmers (Smith et al., 2012; Van Zanten et al., 2014; Fagerholm et al., 2016; Torralba et al., 2016; Udawatta et al., 2019; Wilson and Lovell, 2016; Moreno et al., 2018). Despite the ecological benefits noted, plant production in temperate agroforestry systems poses challenges for agronomists and horticultural farmers. This is even more characteristic of the MAP (Medicinal and Aromatic Plant) species, since they are subject to special drug quality requirements. Finding answers to the challenges is the task of the multidisciplinary agricultural sciences, to which we contribute our research herewith.

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https://doi.org/10.1016/j.indcrop.2021.113764
Received 23 March 2021; Received in revised form 14 May 2021; Accepted 21 June 2021
Available online 26 June 2021

The demand for medicinal plant-based products in the pharmaceutical, food and cosmetics industries is growing, and is expected to do so in future (Lubbe and Verpoorte, 2011; Bernáth, 2013). The proportion of wild growing and cultivated MAPs is increasingly shifting towards cultivation in the developed countries. The question is whether agroforestry offers the right perspective for adapting cultivated species to a new, eco-friendly production system and introducing wild species into cultivation. Alley-cropping seems to be the most viable cultivation system for large-scale and regenerative production of *MAP species in temperate zone AFS;* on the other hand, permaculture farms and forest gardens are viable alternatives for the changemaker small-scale farmers (Zubay et al., 2019).

Minimizing adverse interspecific interactions (competition for water and nutrients, shade, allelopathy) and maximizing niche separations are essential to the success of plant production in AFS. However, limited science-based information is available on the effects of trees on the cultivation of MAPs in temperate zones (Jose et al., 2004; Batish et al., 2008). One fundamental task of medicinal agroforestry systems is to reveal the light intensity level requirement of different MAPs, where the production of biomolecules can still be optimized according to pharmacopoeial expectations. Based on this knowledge, the most suitable species for production in medicinal-agroforestry systems could be determined. Though ecological interactions form complex networks in agroforestry, in this study we specifically addressed the effect of shade on the production of seven MAP species cultivated in temperate zones, as well as the accumulation of their biologically active compounds. The shade effect is influenced by a wide variety of factors. They are: growth vigour, cutting cycle, vegetation length and cultivation technology of the tree species; the spacing and orientation of the AFS on one side, and the plant life-form and agrotechnical needs of the intercrop on the other side. In general, light may be a major limiting factor in AFS. In the cultivation of MAPs, it is particularly important to achieve the required drug quality in terms of the accumulation of their respective biologically active compounds. Very limited scientific information is available on the relationship between shade tolerance and the accumulation of special metabolites of temperate zone MAPs.

Our question was: are there species of MAPs grown in temperate zones which can produce quality drugs specified in the professional standards, even under shade conditions? Our hypothesis was that the response of MAPs to light reduction is species-specific. By means of a three-year-long screening type field experiment, we attempted to clarify the range of species to be recommended for further large-scale agroforestry research.

2. Materials and methods

2.1. Experimental site and plant material

Seven different MAP species (yarrow - *Achillea collina* Becker, marigold - *Calendula officinalis* L., caraway - *Carum carvi* L., Moldavian dragonhead - *Dracocephalum moldavica* L., lemon balm - *Melissa officinalis* L., basil - *Ocimum basilicum* L., savory - *Satureja hortensis* L.) were studied in a three-year field experiment. The propagation material originated from the gene bank of the Department of Medicinal and Aromatic Plants of Hungarian University of Agriculture and Life Sciences or from commercial companies (Table 1). MAP species were selected to represent species with different drug types and special metabolites. The plants were grown at the Experimental and Research Farm of the University in Budapest, Hungary (47°24′09.5″N 19°08′60.0″E) from March 2018 to August 2020. Hungary is located in the temperate zone, in the Pannonian biogeographical region. The local climate globally is described by significant annual temperature fluctuations and four distinct and alternating seasons. Climatological dataset of the experimental site is shown in Table 2.

All plant accessions used in the experiment were propagated from seeds, either by seedlings or by direct sowing (Table 3), according to the usual agrotechnics (Bernáth, 2013). Seedlings were grown in the greenhouse of the Experimental and Research Farm in peat-containing propagation trays, pricked after the appearance of the first foliage, and after acclimatization, planted in the field. The soil type is sandy with a low humus content (1.35–1.79 %) with a pH of 7.6−7.9. The topsoil is 40 cm deep and the $CaCO_3$ content is between 0.6 % – 0.9 %. The experimental plots were set up in a homogeneous soil area, based on soil testing data. The available nutrient content of the major macro- and micronutrients is as follows: NO_3-N: 6.2 ± 0.5 mg/kg; P_2O_5: 365 ± 112 mg/kg; K_2O: 50.5 ± 9.7 mg/kg; Mg: 33.7 ± 4.8 mg/kg; Fe: 40.4 ± 3.2 mg/kg; Zn: 8.12 ± 0.32 mg/kg; Cu: 3.4 ± 0.26 mg/kg. Management of soil fertility was done with Wuxal Super (4 mL/ 4 L irrigation water/ m^2) before flower initiation of each species. Weed control was done by hoeing. Regular irrigation was applied in the dry periods. Both control and treated plants were harvested by hand at the characteristic, optimal phenological phase of the species.

Table 1
Origin of MAP species.

Scientific name	Common name	Variety	Origin
Achillea collina Becker	yarrow	'Azulenka' Optionally listed medicinal plant variety (code: 376637) in Hungarian National List of Varieties. Most important cultivation characteristics: late flowering; branching shoot system, high plant stock; high yield; high EO (0,29 ± 0,051 ml/100 g DW) and proazulene (0,173 ± 0,052 % D.W.) content (Inotai et al., 2016)	Genebank Department of Medicinal and Aromatic Plants
Calendula officinalis L.	marigold	commercial	Rédei Kertimag Zrt.
Carum carvi L.	caraway	commercial	Hermes ÁFÉSZ
Dracocephalum moldavica L.	Moldavian dragonhead	commercial	Jelitto Staudensamen GmbH
Melissa officinalis L.	lemon balm	'Lemona' Height with flowers: 40 cm Phytochemical characteristics: 0.298 ± 0.02 mL/100 g D.W. EO content; 2.43 ± 0.07 RA % D.W. (Szabó et al., 2016)	Jelitto Staudensamen GmbH
Ocimum basilicum L.	basil	commercial	Hermes ÁFÉSZ
Satureja hortensis L.	savory	commercial	Rédei Kertimag Zrt.

2.2. Experimental design and light interception

Artificial shade conditions were developed as an imitation of shade of trees to evaluate the sole effect of shade independently from the effect of allelopathy and competition for water and nutrients existing in agroforestry systems.

For each species, two shading levels were set up: no shade (control: C), and 30 % shade (treatment 1: T1 - light intensity decrease all day long). In 2019, a 50 % shade (treatment 2: T2 - light intensity decrease all day long) was used in the case of four species (caraway, Moldavian dragonhead, marigold, savory), in order to explore their highest shade-tolerance level. Shade treatments were established using commercially available agro green shade nets (LC Packaging TPI Ltd.) with fabric weight of 35 GSM (g/m^2) on frames. Shade nets were secured and

Table 2
Meteorological data in 2018-2020.

	Soil Temperature [°C]			HC Air Temperature [°C]			Precipitation [mm/day; sum mm/month]			HC Relative Humidity [%]		
	2018	2019	2020	2018	2019	2020	2018	2019	2020	2018	2019	2020
April	13.45	12.61	10.83	15.38	12.70	10.75	0.86 25.8	0.87 26.0	0.61 18.2	66.82	61.25	53.34
May	19.99	14.27	14.01	19.07	13.66	13.91	2.30 71.2	7.36 228.4	0.66 14.0	68.38	79.93	65.31
June	21.40	22.03	18.33	20.81	22.69	20.08	3.24 97.20	2,20 66.0	3,31 96.0	74.11	73.19	77.39
July	21.96	21.66	20.10	21.78	21.10	21.11	2.41 74.6	3.54 109.8	2.17 67.4	71.28	69.30	73.62
August	23.57	21.67	21.15	22.59	22.14	2220	1.30 35.0	1.08 32.8	1,94 60.0	72.57	74.07	73.00

Table 3
Method and time of propagation and the phenophase of harvest.

Species	Technology	Spacing	Time of establishment			Phenophase of harvest	Harvested plant part
			2018	2019	2020		
Achillea collina Becker	Seedling	60 × 30 cm		May	2nd year	full flowering stage	floral horizon (upper 30 cm of the shoot)
Calendula officinalis L.	Direct sowing, thinning	50 cm between rows	March	March	March	Full flowering regularly (every 3–4 days)	inflorescence
Carum carvi L.	Direct sowing	24 cm between rows	April	April	–	Fruit ripening regularly (every 3–4 days)	ripe fruits
Dracocephalum moldavica L.	Seedling	50 × 40 cm	–	May	May	full flowering stage	flowering shoots (with 15 cm stubble)
Melissa officinalis L.	Seedling	60 × 30 cm	May	2nd year	3rd year	appearance of first buds	shoot (with 10 cm stubble)
Ocimum basilicum L.	Seedling	50 × 30 cm	May	–	May	full flowering stage	flowering shoots (with 8 cm stubble)
Satureja hortensis L.	Seedling	40 × 30 cm	May	May	May	full flowering stage	flowering shoots (with 6 cm stubble)

attached vertically to the top of the frames at a height of 3 m above the ground. One layer of shade net was used for treatment 1, whilst two layers were used for treatment 2. The plots were kept clean by mechanical weed control.

The area of the experimental plots was 10 m^2 (per species and per treatment). On each plot, four observation units, interpreted as four biological replicates, were marked for data and sample collection.

2.3. Morphological characteristics and yield

Plant height, plant width, fresh mass and drug mass were measured. The measurements of morphological parameters were carried out in 10 replicates/treatment immediately before harvest. Plant height was measured as the length of the longest shoot from the root neck to the tip of the shoot. Plant diameter was measured as an average natural horizontal expansion of the shoots in the case of marigold, Moldavian dragonhead, basil and savory, whilst the width of one row was measured for yarrow, caraway and lemon balm. Both the height and the width of the plants were determined by use of a tape measure. During sampling, healthy developed plants were selected randomly and collectively as bulk samples from the four observation units, avoiding any border effect.

In the case of yarrow, marigold, caraway and lemon balm, 1 square meter was sampled in 4 replicates/species and per treatment. In the case of Moldavian dragonhead, basil and savory, 4 plants were sampled in 4 replicates/species and per treatment. The plants were harvested in the optimal phenological stage; accordingly there were differences in the harvest times of the shaded and control plots. On average, a 7-day delay in flowering on the shaded plots was registered for yarrow and marigold, a 5-day delay for caraway and lemon balm, and 2 days for basil, whilst no delay was registered in Moldavian dragonhead and savory. The harvesting characteristics are summarized in Table 3. Immediately after harvesting, the fresh plant material was transferred from the plots, and was measured to obtain the amount of fresh mass. The plant parts were dried at room temperature till constant weight and the dry mass was registered.

2.4. Phytochemical measurements

2.4.1. Sample preparation

After drying, the samples of the four sampling units were mixed, creating one bulk sample for each plot. In the case of *Dracocephali herba, Basilici herba* and *Saturejae herba*, flower and leaf parts were separated from the stems and only these were used for distillation. Similarly, the leaves of lemon balm were separated from the stem, while for yarrow, the *herba* was used. All of these samples were chopped and homogenized before analysis. The fruits of caraway were ground to powder. For the flowers of marigold, all the petals were separated from the inflorescences, resulting in the *Calendulae flos sine calycibus* drug, which was homogenized and ground to powder before the extraction of the flavonoids and carotenoids. Three replicates were taken from the homogeneous mass samples, as replicates. Each replicate was measured three times, which resulted in phytochemical parameters. To determine the EO content, each of the three replicates was measured once.

2.4.2. Determination of essential oil content

Except for *Melissae folium,* essential oil (EO) was extracted from 20 g of drug material by hydro-distillation (500 mL water) with a Clevenger-type apparatus for 2 h, according to the method recommended in the Hungarian Pharmacopoeia VIII (Pharmacopoeia Hungarica, 2004). In the case of *Melissae folium,* 15 g of plant material was extracted. The EO content is expressed in mL/100 g dry weight (DW).

2.4.3. Determination of total flavonoid content, rosmarinic acid and proazulene content

The assay of total flavonoid content (TFC) determination was done according to the method given in the Hungarian Pharmacopoeia VIII (Pharmacopoeia Hungarica, 2004) for *Calendulae flos*. In brief, 0.8 g of dried powdered plant material (*Calendulae flos sine calycibus*) was extracted by 1 mL of hexamethylenetetramine, 7 mL of hydrochloric acid and 20 mL of acetone for 30 min, and then it was filtered. Subsequently, the extraction was repeated with 20 mL acetone twice and diluted with water and ethyl acetate. The absorbance was measured at 425 nm in the spectrophotometer (Thermo Evolution 201) after incubation for 30 min and expressed in hyperoside per DW. Compensation liquid was prepared from glacial acetic acid and methanol.

The determination of rosmarinic acid content (RA) was based on the method published by Szabó et al. (2016) as follows: „Extract preparation: 0.5 g powdered dry plant material was suspended in 40 mL ethanol. The suspension was heated for 30 min in a water bath, then it was cooled, and finally filtered (by 45 μm filter) into a 100 mL flask. The filtrate was completed by the same solvent (ethanol) to 50.0 mL volume. Rosmarinic acid content was determined by the HPLC method. The Waters HPLC system consisted of a 1525 binary pump with a 717 plus autosampler, a Jetstream column thermostat and a 2998PDA detector, controlled by Empower2 software. A Kinetex C-18 column was used, 100 mm L 4.6 mm i.d., 2.6 μm particle size. All solvents were HPLC grade. For the elution, 1:19:80 phosphoric acid:acetonitrile:water (mobile phase A) and 1:40:59 phosphoric acid:methanol:acetonitrile (mobile phase B) were used as solvents at a flow rate of 1 mL min^{-1} based on the VIII. European Pharmacopoeia section about *Melissae folium* (Pharmacopoeia Europaea, 2013). The gradient program started at 100 % A and after solvent B was increased linearly and reached 35 % at 10 min, then 100 % at 2 min. Finally, 100 % A was reached at 2–8 min post-time for the equilibration of the initial solvent composition. The column temperature was maintained at 35 °C and the injection volume of 5 μl was used in all experiments."

The proazulene content in the EO samples of *Millefolii herba* was determined by the spectrophotometric method at 608 nm, as described in the European Pharmacopoeia VIII. (Pharmacopoeia Europaea, 2013).

2.4.4. Determination of total carotenoid content

The plant materials were extracted two times with MeOH and once with diethyl ether (Et2O). The three MeOH extracts and the ethereal extract were combined, transferred to a separatory funnel and diluted with Et2O. The ethereal phase was washed free from MeOH with water and dried over anhydrous Na_2SO_4. This solution was saponified with 30 % KOH–MeOH at room temperature overnight (for 18 h). After this process the ethereal solution was washed free from alkali, evaporated to dryness under vacuum and dissolved in benzene. This solution was stored in darkness under nitrogen at −20 °C until further investigations.

The total carotenoid content was estimated spectrophotometrically at 450 nm (E1%1cm = 2300) using a Jasco V-530 Spectrophotometer (Schiedt and Liaaen-Jensen, 1995).

2.5. Statistical analysis

The effects of shade treatments (T1 or T2 vs Control) on the morphological characteristics (plant height and width, [cm]), yield (fresh and dry yield, [g/m2 or g/plant]) and phytochemical measurements (EO [(mL/100 mg dry weight], and TFC, RA or proazulene content [%]) of seven MAP species were analysed in each year (2018, 2019, 2020) by paired Student's t test. The normality of the difference variables was tested by d'Agostino's normality test ($p > 0.05$). To avoid the increase of familywise Type I error, we performed Bonferroni's correction.

3. Results and discussion

Achillea collina Becker.

Shade-grown yarrow plants grew significantly higher during the year they were planted ($t(9) = 8.32$; $p < 0.001$) and also in the second year ($t(9)=2.91$; $p < 0.05$), compared to the control (Table 4.). Considering the first-year-old plantations, an average difference of more than 20 cm occurred, whilst in the two-year-old plots, a mean difference of 10 cm was observed. The reaction of yarrow to the shade was neutral in both years, in terms of horizontal extension; and no significant difference was found ($t(9)>1.58$; $p > 0.05$; Table 4). At first, significant yield increase (fresh yield 2019: $t(3) = 6.61$; $p < 0.05$; dry yield 2019: $t(3)=5.45$; $p < 0.05$) was recorded for the plants grown under shade conditions (30 %), compared to the control. In the case of the two-year-old plants, however, no increment was observed in the weight of either the fresh or dried plants (Table 5). In the year of establishment, shading significantly decreased the EO content of the flowering shoots ($t(2) = 16.00$; $p < 0.01$), which was not found equally significant for the two-year-old plants ($t(2)=3.51$; $p = 0.07$, Table 6). The EO content in the year of planting of the plants grown under 30 % shade did not meet the requirements of the Pharmacopoeia (EO content>0.2 mL/100 g; Pharmacopoeia Hungarica, 2004), (Table 6). With the growth of stock, by the second year, however, the shade (30 %) no longer negatively affected the accumulation of the active substance. The EO content of both the shaded and the control samples yielded adequate results (Table 6). Similarly to the EO, its proazulene content was undoubtedly negatively affected by the shade ($t(2) = 10.58$; $p < 0.05$) in the initial year. Nevertheless, both plots — whether grown under the shade (0.08 mL/100g) or full sun exposure (0.10 mL/100g) — produced proazulene content which did fulfil the Pharmacopoeia standards (proazulene content >0.02 %), (Pharmacopoeia Europaea, 2013), (Table 6). In the second year, the proazulene content in the parcels under shade was found to be significantly ($t(2) = 7.18$; $p < 0.05$) higher (0.14 mL/100g) than in the control (0.12 mL/100 g) (Table 6).

Yarrow is a cultivated MAP with limited cultivation area. It has the basics of cultivation technology but with many practical problems (standard drug quality, chemical weed control). Its breeding goals include the development of a morphologically homogeneous flowering horizon for its optimal machine harvestability. As a result of the 30 % of shade treatment applied in the experiment, the plants grew taller; however, the higher average height was also coupled with higher standard deviations. The height of the plants grown under shade conditions demonstrated a more heterogeneous plot compared to the fully sunlit control plants, which is not advantageous in terms of machine harvestability specifically aimed at excellent drug quality.

Shading (30 %) helped the plants to grow in the year they were planted, and although this no longer played a role in the perennial plants, there was no definitive negative effect either. The above findings comply with those of Giorgi et al. (2014), in the case of *Achillea collina cv.* 'Spak', where the yields were not affected by poorer light supply, but instead, the organ ratios were. Plants grown under shade (30 %) developed a higher leaf mass, but smaller inflorescences compared to those grown in full light. This was also recognized in the first year of the present experiment: 2% of the increase in stem fresh weight, 15.8 % of the increase in leaf fresh weight, 17.9 % of the decrease in inflorescence fresh weight in the shade-grown plants (data not published). The decrease in leaf/inflorescence ratio is unfavourable for yarrow cultivation because it is the inflorescence that accumulates the largest amount of EO, as suggested by Németh et al. (2007). This nonetheless can be compensated by higher biomass production in the case of EO oriented cultivation.

In regard to the active ingredients, we recognized a process opposite to what happened with the yield: the shade effect (30 %), in the first year, reduced both the EO and proazulene accumulation, then in the second year this effect levelled off in terms of the EO content, and contrarily, in the case of proazulene it reversed and increased the

Table 4
Mean and standard deviations of plant height (cm) and plant width (cm) of the examined species (control: C, 30 % shaded: T1 and 50 % shaded: T2) in years 2018, 2019, 2020, together with the treatment effect significance (Student's t test).

	Plant height (cm)				Plant height (cm)		
Species	Treatment	Mean (cm)	Std. Deviation	Sig.	Mean (cm)	Std. Deviation	Sig.
Achillea collina Becker	T1-2019	74.90	6.81	***	42.80	5.79	0.05
	C-2019	50.50	5.34		36.80	5.81	
	T1-2020	77.50	11.55	*	65.70	5.14	0,15
	C-2020	68.60	7.89		68.20	4.52	
Calendula officinalis L.	T1-2018	64.40	8.10	**	66.40	4.40	***
	C-2018	46.50	4.84		43.90	6.67	
	T2-2019	62.50	8.34	0.10	56.70	4.06	0.64
	C-2019	53.90	5.40		55.10	9.17	
	T1-2020	54.50	3.10	*	29.60	4.88	0.40
	C-2020	48.70	4.55		31.60	3.66	
Carum carvi L.	T1-2018	80.50	9.11	***	24.90	2.42	***
	C-2018	57.40	9.23		19.90	2.42	
Dracocephalum moldavica L.	T2-2019	49.40	6.04	0,24	37.90	3.21	***
	C-2019	46.80	7.13		57.40	5.99	
	T1-2020	59.30	5.81	**	46.30	8.07	**
	C-2020	46.20	4.87		31.70	6.29	
Melissa officinalis L.	T1-2018	34.70	6.31	*	60.20	3.26	0.07
	C-2018	39.20	3.71		55.30	7.57	
	T1-2019	43.10	6.51	**	79.30	5.27	0.20
	C-2019	53.50	4.38		75.00	10.51	
	T1-2020	54.30	5.60	*	74.10	4.51	***
	C-2020	49.00	2.54		61.30	2.67	
Ocimum basilicum L.	T1-2018	65.70	5.38	0,12	49.70	3.56	0.06
	C-2018	62.40	4.84		46.40	4.48	
	T1-2020	48.90	5.04	*	40.60	4.01	*
	C-2020	40.80	6.34		45.70	3.09	
Satureja hortensis L.	T1-2018	37.60	5.44	0.84	43.40	7.76	0.30
	C-2018	37.20	2.86		40.20	5.35	
	T2-2019	40.90	2.96	*	32.30	5.48	0.14
	C-2019	44.30	3.09		35.00	3.56	
	T1-2020	34.50	3.54	**	28.80	3.97	*
	C-2020	23.40	4.09		21.50	2.64	

Significant at *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ level.

content. The vegetation period of 2020 was much drier than the growing season of 2019. As a result of the difference in meteorological circumstances, the effect of shade could be positive in the case of dry spring and summer through preservation of the soil water content; on the other hand, the positive effect could be cancelled among the conditions of the wet growing season. Under climatic chamber conditions, the EO and proazulene content of yarrow was neither affected by any difference in light supply nor in temperature (Kindlovits et al., 2014), suggesting that the species has cosmopolitan distribution and demonstrates broad ecological adaptiveness. However, these observations can only be interpreted implicitly for a plant reaction under field conditions. Among the phenoloid type components, both the luteolin-7-O-glucoside and apigenin-7-O-glucoside contents and the TFC showed a certain decrease in the plants grown in the shade, while the antioxidant capacity did not change (Giorgi et al., 2014). Based on the various results of previous findings, it can be suggested that moderate shading has a less significant effect on the cultivation and drug quality of yarrow than the different environmental circumstances of growing years.

Overall, yarrow as a perennial plant could be promising for medicinal agroforestry cultivation. The negative effects experienced in this study (less homogeneous plant stock, decreased content of values in the year of planting) can certainly be expected to be reduced by the planting of an appropriate genotype and further technological optimization.

Calendula officinalis L.

Marigold showed a tendency to grow significantly higher than in the control plot in both years (2018: $t(9) = 5.10$; 2020: $t(9) = 3.58$; $p < 0.05$) when receiving 30 % shade treatment. (Table 4). In the case of 50 % shade, there was no significant effect on the height of plants (2019: $t(9) = 2.51$; $p = 0.10$). In 2018, the width of plants increased significantly as a result of the T1 treatment ($t(9) = 12.07$; $p < 0.001$). On the other hand, it recurred neither during 30 % shade treatment of another year ($t(9) = 0.88$; $p = 0.40$), nor in the period of 50 % shadow treatment ($t(9) = 0.48$; $p = 0.64$, Table 4). In the year 2018, the fresh inflorescence yield grew significantly ($t(3) = 6.02$; $p < 0.01$) due to shade treatment (30 %), although this was no longer reflected as growth concerning dry yield ($t(3) = 3.59$; $p = 0.11$, Table 5). In the following years, no significant difference was detected concerning inflorescence yield, whether it be 30 % or 50 % shade treatment (fresh, 2019, T2: $t(3) = 3.70$; $p = 0.10$, fresh, 2020, T1: $t(3) = 2.06$; $p = 0.40$; dry, 2019, T2: $t(3) = 3.50$; $p = 0.12$, dry, 2020, T1: $t(3) = 0.43$; $p = 0.70$). Overall, shading resulted in favourable trends for the accumulation of biologically active substances (flavonoids, carotenoids) in marigold, although of these, only the increase in total carotenoid content in 2020 proved to be significant ($t(2) = 10.09$; $p < 0.05$) as caused by the decrease in light supply (Table 6). The TFC in all plots reached the pharmacopeial requirement (0.4 %).

The development of marigold under experimental conditions was hardly affected by light supply. Apart from a slight elongation due to shade, there was no unfavourable decrease either in yield or accumulation of biologically active compounds compared to plants grown in full light. Nevertheless, these results partly contradict the claim in the literature that marigold is a very light-demanding species (Gilman and Howe, 1999). It also seems to have a high degree of adaptability, thus reduced light supply (up to 50 %) does not cause significant stress. Accordingly, it may be a potentially beneficial MAP intercrop in medicinal agroforestry cultivation. However, further studies may be recommended to further

Table 5
Mean and standard deviations of fresh yield (†g/m2; g/plant) and drug mass (†g/m^2; g/plant) of the examined species (control: C and 30 % shaded: T1 and 50 % shaded: T2) in years 2018, 2019, 2020, together with the treatment effect significance (Student's t test).

		fresh yield (†g/m2; g/plant)			drug mass (†g/m^2; g/plant)		
Species	Treatment	Mean (†g/m^2; g/plant)	Std. Deviation	Sig.	Mean (†g/m^2; g/plant)	Std. Deviation	Sig.
Achillea collina Becker †	T1-2019	1006.50	162.70	*	300.00	41.34	*
	C-2019	666.50	107.27		211.00	32.68	
	T1-2020	1048.25	19.05	0.07	326.25	10.53	0.08
	C-2020	1257.50	174.25		419.75	60.85	
Calendula officinalis L.†	T1-2018	200.50	9.98	*	30.46	1.41	0.11
	C-2018	139.75	10.37		25.80	1.23	
	T2-2019	197.50	14.20	0.10	36.62	3.23	0.12
	C-2019	263.00	21.60		49.46	4.32	
	T1-2020	119.75	11.50	0.13	20.25	1.89	0.70
	C-2020	100.25	13.94		19.25	3.59	
Carum carvi L.†	T1-2018				18.88	2.08	**
	C-2018				3.96	0.41	
	T2-2019				2.41	0.44	*
	C-2019				4.79	0.82	
Dracocephalum moldavica L.	T2-2019	54.75	7.18	*	28.00	4.38	*
	C-2019	131.50	28.90		65.38	10.14	
	T1-2020	160.25	30.58	*	128.00	26.58	*
	C-2020	75.25	13.30		62.25	11.15	
Melissa officinalis L.†	T1-2018	889.00	82.21	0.06	190.75	26.25	0.08
	C-2018	584.00	84.11		144.00	17.28	
	T1-2019	894.00	78.01	0.30	143.25	12.04	0.46
	C-2019	805.50	124.91		127.00	41.84	
	T1-2020	1503.00	152.22	*	391.50	42.81	*
	C-2020	1048.00	149.99		219.00	40.18	
Ocimum basilicum L.	T1-2018	211.45	17.94	0.06	31.84	3.47	0.21
	C-2018	182.93	14.56		29.25	2.78	
	T1-2020	153.25	32.21	*	27.50	5.07	*
	C-2020	320.25	38.06		58.75	5.74	
Satureja hortensis L.	T1-2018	81.72	5.66	0.10	18.13	2.42	0.85
	C-2018	71.44	3.94		17.72	1.73	
	T2-2019	93.13	10.35	*	11.50	2.04	**
	C-2019	124.75	15.82		19.69	2.72	
	T1-2020	78.88	11.375	0.25	19.25	3.59	0.42
	C-2020	70.00	16.068		17.13	6.43	

Significant at *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ level.

demonstrate a high degree of shade tolerance under different soil and microclimate conditions.

Carum carvi L.

Height and width data could only be recorded in 2018; as a result of the 50 % shade in 2019, the plants in the treatment parcel tumbled to such an extent that it was not possible to measure them. Due to the 30 % shade treatment, both plant height ($t(9) = 4.91$; $p < 0.001$), and width ($t(9)=5.00$; $p < 0.001$) increased significantly. The yield was monitored and recorded in both years. The 30 % shade produced a significantly noteworthy increase in yield (T1: 18.88 g/m^2; C: 3.96 g/m^2; $t(3) = 13.46$; $p < 0.01$), on the contrary, the yield decreased significantly - to about half – due to the 50 % shade treatment ($t(3)=6.37$; $p < 0.05$, Table 5). Shade treatments of any degree had a significant negative effect on EO accumulation ($t(2)>6.70$; $p < 0.05$). Nevertheless, considering the experimental conditions and the variety applied, the EO content (4.98 mL/100 g and 3.677 mL/100g, respectively) of plants under either 30 or 50 % of shade still managed to meet unquestionably the pharmacopeial quality requirements (3 mL/100g) (Table 6).

Caraway is a MAP species that can be cultivated using large-scale cereal technology. Its cultivation has a well mechanised technology and can therefore be cultivated in a silvoarable type of AFS. Based on the yield response of caraway to the effect of shade in these experiments, the limit range of light demand/shade tolerance is outlined. With this deeper knowledge, the agroforestry cultivation technology of caraway can be optimized. Under the humus-poor, easily drying, weak sandy soil conditions of the experimental area, the caraway responded to the 30 % shade treatment with a multiple yield increase, which can be potentially explained by the indirect effects of shade (temperature equalization, reduction of evapotranspiration). However, a decrease in light supply at an even larger degree proved to be a stress effect, to which the initial reactions were abnormal elongation, weakening of the shoot and decrease in yield. Besides, the reduced light supply resulted in a 0.68–1.47 % lower EO content, which appears to be consistent with the findings of Bouwmeester et al. (1995). On the other hand, depending on the variety and the purpose of cultivation, this reduction does not necessarily result in poor drug quality. Generally the continuation of caraway experiments for medicinal agroforestry cultivation could further clarify the interactions between shading, other ecological factors and economic conditions.

Dracocephalum moldavica L.

As a result of the 30 % shade treatment, dragonheads grew significantly higher ($t(9) = 4.17$; $p < 0.01$), and wider ($t(9)=3.80$; $p<0.01$) whilst the 50 % shade treatment did not seem to affect the height ($t(9) = 1.26$; $p = 0.24$), but decreased the horizontal growth significantly ($t(9)= 9.97$; $p < 0.001$, Table 4). The 30 % reduction in light multiplied both the fresh (T1: 160.25 g/plant; C: 75.25 g/plant; $t(3) = 5.44$; $p < 0.05$) and dry yields (T1: 128.00 g/plant; C: 62.25 g/plant; $t(3)=5.11$; $p < 0.05$) compared to the results for plants under full sunlight (Table 5). On the other hand, both fresh yield (T2: 54.75 g/plant; C: 131.50 g/plant; $t(3) = 4.78$; $p < 0.05$) and dry yield (T2: 28.00 g/ plant; C: 65.38 g/plant;

Table 6
Mean and standard deviations of biologically active compounds (mL/100 mg dry weight for EO; %) of the examined species (control: C and 30 % shaded: T1 and 50 % shaded: T2) in years 2018, 2019, 2020, together with the treatment effect significance (Student's t test).

Species	Biologically Active Compound	Treatment	Mean (%)	Limit (Ph. Eur.)	Std. Deviation	Sig.
Achillea collina Becker	EO (ml/100 g DW)	T1-2019	0.19	>0.2 ml/100 g	0.01	**
		C-2019	0.30		0.01	
		T1-2020	0.28		0.00	0.07
		C-2020	0.30		0.01	
	proazulene	T1-2019	0.08	>0.02%	0.00	*
		C-2019	0.10		0.00	
		T1-2020	0.14		0.00	*
		C-2020	0.12		0.00	
Calendula officinalis L.	carotenoid	T2-2019	1.62		0.05	0.07
		C-2019	1.38		0.03	
		T1-2020	2.43		0.07	*
		C-2020	2.04		0.03	
	flavonoid	T1-2018	0.66	>0.4 %	0.10	0.96
		C-2018	0.66		0.01	
		T2-2019	0.85		0.12	0.35
		C-2019	0.92		0.07	
		T1-2020	1.33		0.02	0.19
		C-2020	1.43		0.09	
Carum carvi L.	EO (ml/100 g DW)	T1-2018	4.98	>3 ml/ 100 g	0.11	*
		C-2018	6.45		0.44	
		T2-2019	3.68		0.12	**
		C-2019	4.36		0.11	
Dracocephalum moldavica L.	EO (ml/100 g DW)	T2-2019	0.71		0.03	0.20
		C-2019	0.80		0.09	
		T1-2020	0.98		0.03	0.06
		C-2020	1.05		0.06	
Melissa officinalis L.	rosmarinic acid	T1-2019	2.71	>1%	0.01	*
		C-2019	2.95		0.05	
		T1-2020	3.71		0.14	*
		C-2020	3.17		0.02	
Ocimum basilicum L.	EO (ml/100 g DW)	T1-2018	1.26		0.06	0.77
		C-2018	1.28		0.09	
		T1-2020	0.90		0.01	0.13
		C-2020	0.83		0.04	
Satureja hortens L.	EO (ml/100 g DW)	T1-2018	4.02		0.12	**
		C-2018	3.53		0.10	
		T2-2019	3.95		0.26	0.07
		C-2019	3.42		0.00	
		T1-2020	5.31		0.06	*
		C-2020	4.53		0.08	

Significant at $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ level; significantly higher is in bold.

$t(3) = 5.51$; $p < 0.05$) were proved to decrease with 50 % less light. Moreover, the essential oil content was not affected by shading ($t(2) < 4.05$; $p > 0.05$, Table 6).

Dragonhead is native to temperate climate regions of Asia but has also been successfully cultivated and introduced to cultivation in countries with a European temperate climate and North African countries, as well as in the United States (Acimovic et al., 2018). *Herba* and *aetheroleum* as the drugs of the plant are used extensively in the spice trade, aroma chemicals market, perfumery, beverage industry and the pharmaceutical industry due to its scent. It contains remarkable notes of lemon balm (neral, geranial), and has beneficial biological properties. Besides, it also adapts better to conditions in colder climate regions, compared to lemon balm. (Galambosi et al., 1989). The latter data suggested that it could perhaps adapt to the shaded conditions located in agroforestry systems; and we confirmed this with the results of the present research. Under our experimental conditions, dragonhead demonstrated a high tolerance level, and furthermore, responded favourably to moderate shade effect with its yield values, without any further reduction in its active ingredients. Since dragonhead also tolerates the allelopathic effects of *Populus tremula* and *Juglans regia* species (Zubay et al., 2021) it is suggested that it may be beneficial in medicinal agroforestry cultivation. For technological advances, therefore, large-scale experiments are recommended.

Melissa officinalis L.

In both the year of planting and the following year the height of lemon balm was significantly reduced ($t(9) = 3.41$; $p < 0.05$; $t(9)=5.56$; $p < 0.01$) by shading (30 %), yet in the third year all plants under shade grew significantly higher ($t(3)=2.99$; $p < 0.05$) than those within the control. There was no significant difference in the width of the plants in the first two years ($t(3)=2.99$; $p < 0.05$), then for the third year similarly to the height of the plant, the 30 % shade treatment increased the width ($t(9)=10.67$; $p < 0.001$), at an average of 13 cm (Table 4). Correspondingly, yields were only affected by the treatment in the third year, also. In 2020, the shaded (30 %) plot resulted in significantly higher fresh yield (T1: 1503.00 g/m^2; C: 1048 g/m^2; $t(3) = 5.02$; $p < 0.05$) and dry yield (T1: 391.50 g/m^2; C: 219.00 g/m^2; $t(3) = 6.85$; $p < 0.05$), compared to those in the control plot (Table 5). In the first year, the EO content became significantly higher in total sunlight (T1: 0.603 ml/100 g; C: 0.770 ml/100 g; $t(2) = 7.62$; $p = 0.05$). In the second year, the shade treatment was less outstanding than the year before ($t(2)=2.11$; $p = 0.17$); yet in the third year, the EO content of the shaded plants (0.682 ml/100g) resulted in a much higher number than that of the control (0.544 ml/100g, $t(2)=119.51$; $p < 0.001$, Table 6). Considering the drug properties of lemon balm, pharmacopeial specification states a value of rosmarinic acid content reaching the minimum of 1% DW, which was undoubtedly achieved by all the samples of this experiment. The aftermath of the treatments happened to be similar both in production and EO content results. In 2019, the decrease in light supply also had a reducing effect on the rosmarinic acid content (T1: 2.71 %; C: 2.95 %), (t (2) = 8.58; $p < 0.05$), whilst in the same plot under shade in 2020 it significantly exceeded (t(2)=7.70; $p < 0.05$) that in the control (3.17 %), (Table 6).

2019 was a warm and rainy year, while 2020 was a cooler and drier year (Table 2), which had an effect on the sandy soil of the experimental site even with regular irrigation. Based on previous results, the 'Lemona' variety is sensitive to drought stress and accumulates significantly less EO in drought stress conditions (Szabó et al., 2017). The sensitivity of the 'Lemona' variety to drought stress is also indicated by our experiment, as it accumulated the least EO in the driest year of 2020. In this dry year, the accumulation of EO in the shaded plot increased compared to the control, which is related to the soil moisture retaining effect of the shading. The EO accumulation was highest in the 2019 rainy year, however, there was no significant difference between the treated and control plots. It follows from all this that a slight shadow (30 %) can buffer the effect of a lack of precipitation. According to literature data (Politycka and Seidler-Łozykowska, 2009; Nurzyńska-Wierdak et al., 2014), the age of the plant does not affect the EO content of lemon balm harvested in the same phenophase, which confirms that lower levels of EO accumulation in the third year of our experiment are indeed the effect of a drier year and are independent of the age of the plants.

Lemon balm is a medicinal and aromatic plant grown throughout Europe, the pharmacological use of which has been intensively researched (Seidler-Łożykowska et al., 2013; Shakeri et al., 2016). The presumed shade tolerance of lemon balm (Oliveira et al., 2016; Russo and Honermeier, 2017), alongside the developed cultivation technologies, and value-added properties such as honey-bearing capacity therefore make it a candidate for medicinal agroforestry cultivation. According to our results, the 30 % light supply reduction influenced all quantitative and qualitative characteristics as a function of the age of the plants and became more favourable with age as well. This phenomenon is supposedly the consequence of complex adaptation, which is worth proving with further research. It would be useful to consider additional genotypes and years, too, so that it may be introduced into medicinal agroforestry cultivation.

Ocimum basilicum L.

The effect of shading (30 %) on the height of basil plants did not emerge in the first year, while a lengthening of the plant was caused (when we repeated the experiment) by the decrease in light supply ($p =$ 0.022) (t(9)=1.74; $p = 0.12$; t(9)=2.75; $p < 0.05$, Table 4). This is also indicated by the width data. Shading decreased the width of the plants only in the second experiment (Table 4). Consistent with this, there was no significant difference in either fresh or dry yield between shaded or plants grown in the sun — in the first year (t(3) = 3.97; $p = 0.06$; t(3)= 1.58; $p = 0.21$). In 2020, the reduction of light supply caused a significant decrease in both fresh yield (T1: 153.25 g/plant; C: 320.25 g/plant; t(3)=4.90; $p < 0.05$) and dry yield (T1: 27.50 g/plant; C: 58.75 g/plant; t (3)=6.31; $p < 0.05$) (Table 5). The EO accumulation was not significantly affected by the shade in any of the years of the study (2018: t(2) = 0.34; $p = 0.77$; 2020: t(2)=2.52; $p = 0.13$, Table 6).

Basil is a MAP native in subtropics that has been successfully grown even in temperate conditions. Its biologically active substances are used (and researched) in the food industry, in medicine, and in plant protection (Shahrajabian et al., 2020; Oxenham et al., 2005). The benefits of intercropping with other species have been described in several studies (Kordi et al., 2020; Pereira et al., 2015; Song et al., 2010). We conclude from our current research that slight shade (30 %) may negatively affect the yield; however, it certainly does not have a significant impact on the accumulation of EO. Research undertaken in Serbia (temperate/ dry continental climate with Mediterranean influence) and the United Kingdom (temperate/ oceanic climate) yielded different results. In the case of climate such as the Serbian, it is suggested that shade (50 %) can improve the accumulation of EO in field conditions (Milenković et al., 2019). Nevertheless, for young basil plants grown in greenhouses in the UK, all shade (22–75 %) treatments significantly reduced both the EO content and the ratio of major components (linalool, eugenol) compared to control plants (Xianmin et al., 2008). Under our test conditions, the two different meteorological conditions of the tested years (year 2018 was warmer and had more precipitation than year 2020) also reflect the hypothesized role of the given ecological conditions in the results. From all this, we presume that the cultivation of basil in medicinal agroforestry is promising in areas where Mediterranean weather conditions occur during the vegetation period. Nevertheless, this may require additional data which take into account the variety, the soil and the intended use of the plant itself.

Satureja hortensis L.

In 2018, the growth of summer savory was not affected by the 30 % shade treatment in either a vertical or horizontal direction (t(9) = 0.21; $p = 0.84$; t(9)=1.10; $p = 0.30$). In 2020, however, a significant effect was observed for both morphological parameters with shaded (30 %) plants significantly higher (34.5 cm; t(9)=5.37; $p < 0.01$) and wider (28.8 cm; t (9)=3.69; $p < 0.05$) compared to the control subjects (23.40 cm; 21.50 cm) (Table 4). In 2019, the treated plot under 50 % shade elongated and grew significantly higher than the control (t(9) = 3.25; $p < 0.05$), (Table 4). The 30 % reduction in light supply was not associated with a significant decrease in yield for either fresh or dry yield (fresh: t(3)< 2.31; $p > 0.10$; dry: t(3)<0.95; $p > 0.40$); however, the 50 % shade treatment reduced both parameters significantly (fresh: t(3)=6.11; $p <$ 0.05; dry: t(3)=13.12; $p < 0.01$). The EO content of the flowering shoots under 30 % shade increased quite significantly (2018: t(2)=27.59; $p <$ 0.01; 2020: t(2)=10.09; $p < 0.05$) in both years (2018: 4.017 ml/100g; 2020: 5.312 ml/100g), compared to the control plots (2018: 3.530 ml/ 100g; 2020: 4.530 ml/100g). According to our data, the EO content did not decrease significantly even during the 50 % shading (t(2)=3.55; $p =$ 0.07, Table 6).

Summer savory is cultivated in several areas of Europe and used worldwide due to its antioxidant, antimicrobial, antiparasitic, pesticidal, anti-inflammatory and hepatoprotective properties (Fierascu et al., 2018). The 30 % shade did not affect the yield of *Saturejae herba* negatively, and at the same time, it did have positive effects on its EO content instead. The latter result is indeed one of the most important findings of our three-year research. In cultivation, summer savory is considered a drought stress tolerant plant (Baher et al., 2002; Radácsi et al., 2016), but it can now also be labelled mild shade tolerant. Therefore, it seems that we can suggest medicinal agroforestry cultivation of summer savory in a temperate zone, because the moderate shade provided by the trees (30 %) may potentially increase the EO yield per unit area, compared to the monoculture. However, scale-up experiments are obviously necessary.

4. Conclusion

In this study, we assessed the response of seven different medicinal and aromatic plants to shade, based on which our main conclusions are the following:

1 We proved that there are temperate grown species of MAPs for which it is possible to produce the quality drug specified in the professional standards and with adequate yields even under shade conditions.
2 Our hypothesis was confirmed: MAPs respond species-specifically to 30 % light reduction. Based on the specificity of these species, in the case of fields with poor soil conditions, as well as setting up scale-up cultivation experiments, we recommend the species mentioned below for medicinal agroforestry cultivation for the following reasons. Marigold: drug yield was not decreased by mild shadow (30 %); however, it increased the carotenoid content. Moldavian dragonhead: mild shade (30 %) increased *Dracocephali herba* yield while not decreasing its EO content. Lemon balm: drug yield can be increased by mild shade (30 %). Savory: mild shade (30 %) did not decrease drug yield but increased its EO content.

Species less tolerant of the 30 % shade are recommended for further agroforestry research with the following suggestions considering research topic orientation: *Achillea collina* Becker: screening of genotypes most tolerant of reduced light conditions (maintaining organ proportions, homogeneous floral horizon). Caraway: setting up experiments for both variety and nutrient replenishment targeting the improvement of EO accumulation; determining the optimal economic centre of gravity for increasing yield and decreasing EO accumulation due to shade. Basil: exploring the optimization of cultivation for light conditions typical of the local agroforestry systems (very mild shade testing).

3 Mild shade (30 %) exerted favourable effects on several examined MAP species of a variety of experimental parameters; however, the 50 % shade effect, conversely, had a favourable effect on none of these examined MAPs of any of the examined parameters. Consequently, in the case of medicinal agroforestry land use in temperate zones, trees must be selected and planted in such a way that the shade effect does not exceed 30 %.

Credit author statement

P. Zubay accomplished the experiment and wrote the manuscript.

K. Ruttner supervised and helped the laboratory work of P. Zubay.

M. Ladányi made the statistical analysis and wrote the statistical parts of the manuscript.

J. Deli made the total carotenoid content measurement.

É. Németh-Zámboriné discussed the manuscript with P. Zubay with several good suggestions and corrections.

K. Szabó wrote the project proposal, supervised the work of P. Zubay during the experiment and manuscript writing.

Declaration of Competing Interest

The authors wish to confirm that there are no known conflicts of interest associated with this work and there has been no significant support for this publication that could have influenced its outcome.

Acknowledgements

Supported by the ÚNKP-2020 New National Excellence Program of The Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund.

This research was supported by the Ministry for Innovation and Technology within the framework of the Thematic Excellence Programme 2020- Institutional Excellence Subprogram (TKP2020-IKA-12) for research on plant breeding and plant protection.

This research was supported by the Ministry for Innovation and Technology within the framework of the Higher Education Institutional Excellence Program (NKFIH-1159-6/2019) in the scope of plant breeding and plant protection research of Szent István University.

Prepared with the professional support of the Doctoral Student Scholarship Program of the Co-operative Doctoral Program of the Ministry of Innovation and Technology financed from the National Research, Development and Innovation Fund.

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