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Impact of hemp (*Cannabis sativa* L.) variety on the seed and stem yield, biochemical characteristics of the inflorescences and nutritional quality of seeds

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Abstract: Hemp is becoming increasingly popular, and many new varieties are coming onto the market to meet the requirements of different industries. In this study, the seed and stem yield, seed nutritional properties and the biochemical characteristics of the inflorescences of seven European varieties (Fedora 17, Futura 75, KC Dóra, Monoica, Santhica 27, Tiborszallasi, USO 31) were investigated in a 3-year field trial. Futura 75 and Tiborszallasi stand out as varieties with the highest potential in the conditions of the experiment (humid continental climate with oceanic influences, heavy soil). Futura 75 achieved the highest seed yield (505 kg/ha dry matter), stem yield (8 036 kg/ha fresh matter), protein yield (140 kg/ha) and oil yield (181 kg/ha). There were no differences in protein content (average 21.0%) among varieties. The total unsaturated fatty acid content was as high as 87.6% at Tiborszallasi. The best ratio between omega-6 and omega-3 fatty acids was 3:1 in Tiborszallasi, which had also the highest oil content (30.2%), the highest total phenolic content (2.8 mg caffeic acid (CA)/g) and the best antioxidant potential (6.69 EC_{50} DPPH (2,2-diphenyl-1-picrylhydrazyl) mg/L). Most varieties had higher cannabidiol and tetrahydrocannabinol contents in the inflorescence at seed maturity (from 0.22 to 3.3 for cannabidiol (CBD) and from 0.00 to 0.32 for tetrahydrocannabinol (THC)) compared to full flowering (from 0.17 to 4.33 for CBD and from 0.00 to 0.52 for THC, on average 2.64% for CBD and 0.19% for THC), presenting an opportunity for dual-purpose use.

Keywords: agronomic performance; varietal comparison; phytochemical profiling; fatty acid composition; antioxidant capacity; cannabinoid profile

Hemp (*Cannabis sativa* L.), a herbaceous plant belonging to the Cannabaceae family, is becoming increasingly popular, and many new varieties with low THC levels (so called "industrial hemp") are coming onto the market to meet the requirements of different industries. The largest cultivation areas in Slovenia are predominantly sown with the French and Hungarian varieties, which are intended for seed and biomass production. French varieties such as Futura 75, Fedora 17, Santhica 70, and Santhica 27 accounted for the largest share of sown areas, 47% in 2021, 56% in 2022, and 32% in 2023 (Korošec 2024),

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among Hungarian varieties are the most dominant Monoica, and KC Dora.

Hemp seeds are recognised as a highly nutritious food source rich in protein and oil with a favourable amino and fatty acid composition and have gained popularity in the food, feed and cosmetics industries. They consist of about 30% oil and 25% protein, both of high nutritional quality, and 10–15% insoluble fiber (Callaway 2004). Hemp proteins have a high nutritional value, as they are rich in several essential amino acids, including arginine, and the sulfur-containing amino acids methionine and cysteine (Callaway 2004, Aluko 2017). Hemp oil is particularly rich in polyunsaturated fatty acids, with oleic acid (OA), linoleic acid (LA) and α-linolenic acid (LNA) being the most important omega-9, omega-6 and omega-3 fatty acids, respectively (Aluko 2017), and plays an important role in the human diet, especially due to the ratio between omega-6 and omega-3 in a desirable range between 2:1 and 3:1 (Callaway 2004). The additional presence of gamma-linolenic acid (GLA) in hemp seed oil ultimately makes its nutritional value better than that of most comparable seed oils. The protein and oil composition of hemp varies by variety, with some having higher levels of certain amino and fatty acids than others. Analysing the protein and oil composition of hemp seed is critical to determining the nutritional value and potential uses of this versatile plant grown in different environments.

Another important bioactive compound from the secondary metabolite family found in hemp seeds (whole seeds, hulls or cake) are phenolic compounds, which show some remarkable biological activities, including anticancer, anti-inflammatory, anti-neuroinflammatory and antioxidant properties (Chen et al. 2012, Martinez et al. 2020). When hemp is grown for a dual purpose, e.g., for seed and cannabinoids, the cannabinoid content in the rape flowers is an important parameter for selecting the best variety.

One of the most important agronomic management factors that influences the overall productivity of hemp is the sowing density, which not only affects weed infestation but also strongly influences the morphological development of the hemp plant. At a higher sowing density, plants close their leaf canopy faster and can compete better with weeds. In general, a lower seeding density is used for hemp plants grown for seed production and a higher seeding density is used for stem (fiber) production. Different seeding densities have been tested in the literature, ranging from very low (50 plants/m^2) to very high seeding densities (750 plants/m^2) (Amaducci et al. 2015, Yazici 2023), but the optimal planting density for each variety should be determined considering the end use of the plant, the type of variety and the soil conditions.

It is important that growers choose the right variety for their specific needs and environmental conditions to optimise seed yield and overall crop productivity. Studies have shown that different hemp varieties can produce different seed yields depending on factors such as environmental conditions, soil quality and cultivation practices (Amaducci et al. 2015). Also, studies on hemp cultivation have shown that the nutritional composition of hemp seeds strongly depends on the variety and agrotechnical factors (Galasso et al. 2016).

The study aimed to evaluate the agronomic performance, nutritional value of the seeds (protein and oil content, fatty acid composition), polyphenol content, antioxidant potential and cannabinoid content of seven European hemp varieties that have been most commonly cultivated in Slovenia in recent years. We hypothesised that the varieties differ significantly depending on their origin. The goal was to select suitable varieties for cultivation in our climate and that could serve as the basis for hemp breeding programs in Slovenia.

MATERIAL AND METHODS

Investigated hemp varieties. The field trial was conducted with seven hemp varieties that have been the most commonly grown varieties in Slovenia in recent years. Varieties for combined use/purpose still predominate. All varieties tested in the experiment are listed in the Common Catalogue of Varieties of Agricultural Plant Species and are frequently used in field trials in European countries (Table 1). The varieties represent diverse agro-climatic origins across Europe, including Atlantic (France), continental (Hungary, Balkans), and Central/Eastern regions (Ukraine, Poland). All are registered EU-certified industrial hemp varieties (less than 0.2% THC), encompassing both monoecious and diecious types commonly cultivated for seed and dual-purpose production.

Field experimental design and evaluation. The trial was designed as a randomised complete block trial with three replicates. The trial area was 18 m^2 (6 × 3 m), with 7 hemp varieties and 2 seed densi-

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Table 1. Origin, sexual type, maturity group and application type of hemp varieties used in the three-year field trial

Variety	Origin	Sexual type	Maturity group	Application
Fedora 17	France	monoecious	early	seed/CBD/fiber
Futura 75	France	monoecious	late	seed/CBD/fiber
KC Dóra	Hungary	dioecious	late	seed/CBD/fiber
Monoica	Hungary	dioecious	medium	seed/CBD/fiber
Santhica 27	France	monoecious	medium	seed/fiber
Tiborszallasi	Hungary	dioecious	late	seed/fiber
USO 31	Ukraine	monoecious	early	seed/fiber

Early < 125 days; medium < 135 days; late < 145 days; CBD – cannabidiol

ties (SD): (*i*) 200 viable seeds/m^2 (SD1) and (*ii*) 150 viable seeds/m^2 (SD2). Different hemp varieties exhibit distinct architecture and canopy structures, so their optimal sowing densities may vary. Higher densities typically promote greater stem elongation and may reduce branching, testing both reflects real practices for hemp in Europe. The row spacing was 12.5 cm and was the same for both planting densities.

At harvest, the plants from the inner 4 m^2 were collected from each plot to determine the seed and stem yield. The plants from 1 m^2 per plot were weighed to determine the yield of fresh stems. Twenty-five plants per sexual type (male, female and/or monoecious) of the same square meter were randomly selected for height determination. For dioecious varieties, the average height of males and females was calculated. Seed moisture and 1 000-seed weight (TSW) were determined according to the protocol of the International Seed Testing Association (ISTA 2005). The seed yield was calculated in kg dry weight per hectare.

The sampling of hemp inflorescences to determine the cannabinoid content was carried out in 2019. The influence of seed density was not considered a factor for the study of cannabinoid content in the inflorescences of female/monoecious plants. Therefore, for each variety, 50 inflorescences/plot were randomly sampled from SD1 or SD2. Three samples per variety were collected at two different times: (*i*) at full flowering of female plants, which occurred on August 5 for all monoecious varieties (except Futura 75) and on August 14 for all dioecious varieties, including Futura 75; and (*ii*) at seed harvest, which occurred on September 19 for USO 31 and Fedora 17, on September 24/25 for Santhica 27, Futura 75 and Monoica, and on September 27 for Tiborszallasi and KC Dora. The samples were dried at 40 °C until a constant mass was reached to determine the cannabinoid content (Figure 1).

Soil characteristics and trail maintenance. The field trials were conducted in 2017–2019 in Ljubljana, Slovenia (46°3'N, 14°30'E; altitude 295 m a.s.l.). The soil at the study site was a hydromeliorated, medium-deep soil with a texture of 25% sand, 42.6% silt and 32.4% clay (Clay Loam; heavy soil). At the beginning of the experiment in 2017, soil analysis showed a pH of 6.8, 129.8 mg P/kg (well-supplied), and 132.0 mg K/kg (moderately supplied) based on Al method (Egner et al. 1960).

The previous crop was soybean in the first year, buckwheat in the second year and peas in the third year. Each year, the residues of the previous crops were plowed under in the fall, and the field was left fallow over the winter. Before sowing, the field was fertilised with 500 kg/ha PK 0:14:28 and 260 kg/ha calcium ammonium nitrate (27% N) and plowed once for seedbed preparation. No additional fertilisers were used. No pesticides were used to control weeds, diseases or pests.

Sowing was carried out on May 19, 2017, May 29, 2018, and June 13, 2019, with a Wintersteiger plot seeder to a sowing depth of 2–3 cm. During seed ripening, a hail protection net was used to protect against birds, so no seed was lost due to bird attacks. Harvest was carried out manually on September 29, 2017, September 27, 2018, and September 19–29, 2019.

Weather conditions during experiment. Figure 2 shows the weather conditions at the time of the field trial and for the 30-year period.

Weather conditions during experimental years deviated from the 30-year average (Figure 2) and likely affected also crop emergence and growth. In 2017, May was colder and drier, followed by a warm summer with a wet June and drier-than-average July and August, and a wet (347 mm rainfall), cool September. In 2018, excessive rainfall immediately

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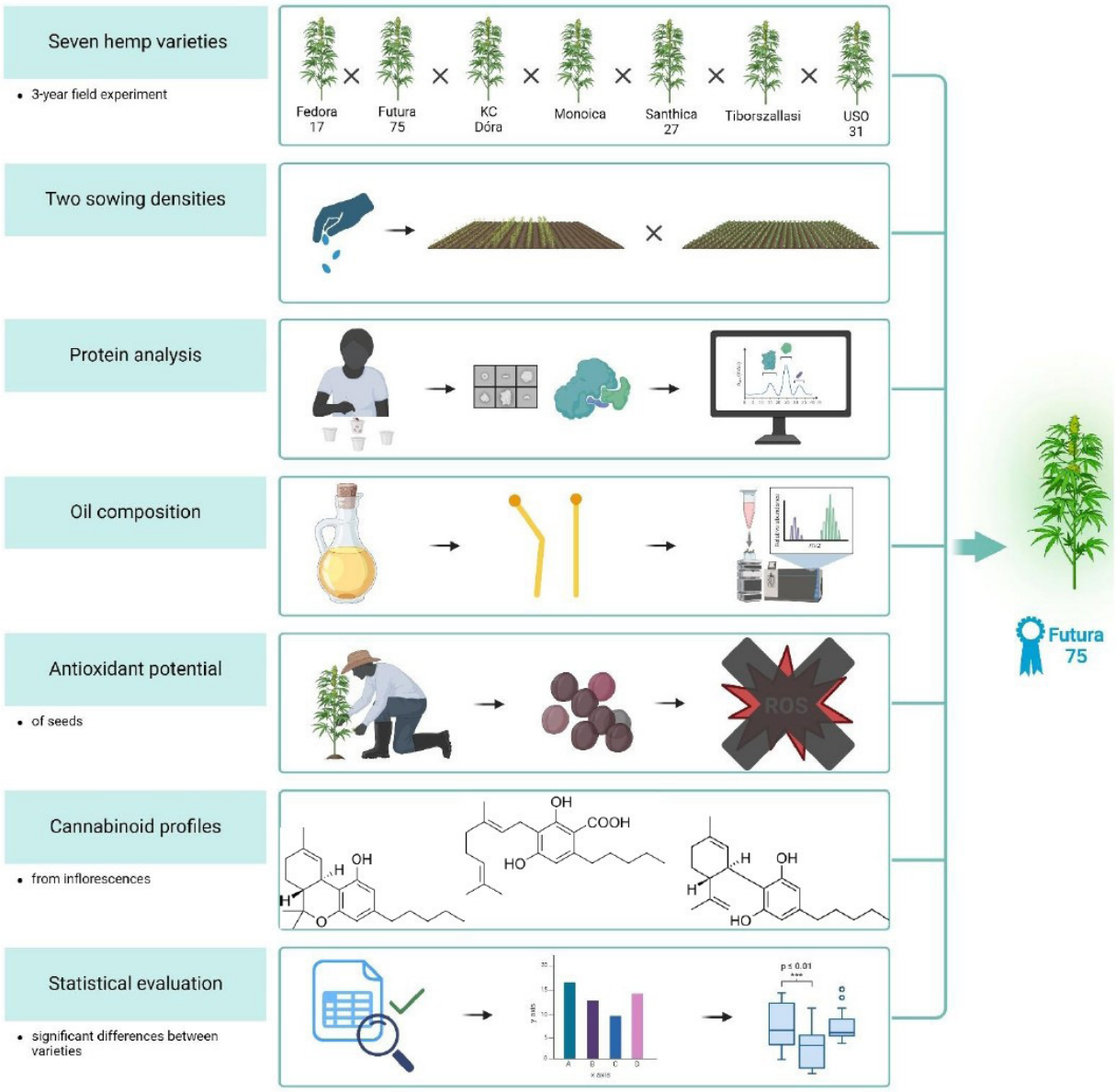

Figure 1. Experiment presentation

after sowing (> 50 mm) likely impaired emergence. Summer months were warmer than average and had above-average rainfall. In 2019, a cold (4.3 °C colder than the 30-year average), wet May (239 mm) was followed by a hot (46 mm) and dry June and a warm summer with irregular precipitation, ending with exceptionally warm, dry September.

Biochemical analysis – analysis of protein and oil content in seed and oil composition. Prior to biochemical analysis of the seeds, the moisture content was determined according to SIST EN ISO 665:2001. The nitrogen content in the hemp seeds was determined using the Kjeldahl method. The oil content was determined using SIST EN ISO 659:1998. The oil and protein contents were calculated by multiplying the dry weight of the seeds by the protein content. The qualitative and quantitative composition of fatty acids was analysed by the preparation of methyl esters of fatty acids according to SIST EN ISO 5509:2001 and by gas chromatographic analysis of methyl esters of fatty acids according to SIST EN ISO 5508:1995.

Biochemical analysis – analysis of polyphenols and antioxidant potential of seed

Extraction of phenolic compounds. To extract the oil, the hemp seeds were crushed in a seed mill

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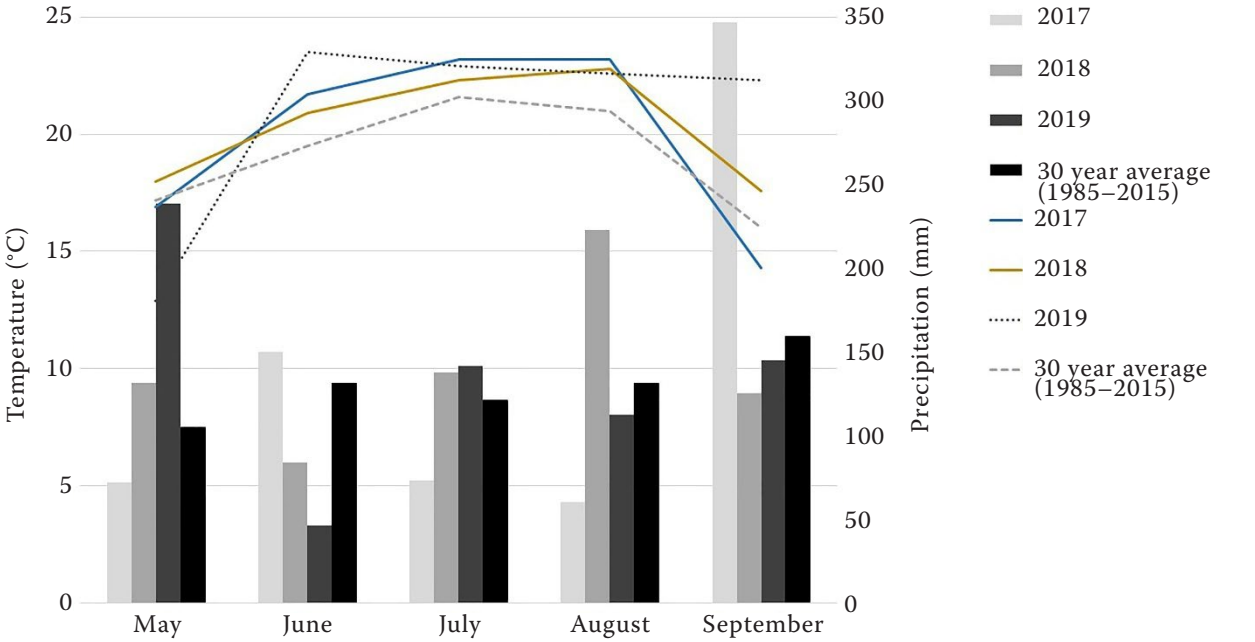

Figure 2. Average monthly temperatures and precipitation for months in the period of the field trial for the years 2017–2019 and for the period 1985–2015 in Ljubljana

under N2 and mixed with hexane (seed to solvent ratio, 1:2.5; *w*:*v*) on a shaker (Vibromix 314 EVT; Tehnica, Železniki, Slovenia) for 10 min at room temperature. The resulting mixture was then centrifuged at 4 000 rpm for 4 min. The supernatant was removed. Hexane was added to the residue, and the procedure was repeated twice. The defatted residue was air dried overnight to remove the remaining hexane. Extraction was carried out with 70% (*v*:*v*) ethanol, with the ratio of defatted seeds to solvent being 1:5 (*w*:*v*). Extraction was carried out according to the following scheme. After 2 h of shaking at room temperature, ultrasound was applied for 30 min without a shaker, followed by 2 h of shaking at room temperature. Afterwards, the extraction mixture was centrifuged at 6 000 rpm for 10 min. The supernatant, i.e., the ethanolic extract, was used to determine the total phenolics and antioxidant activities. During the period of analysis, the extract was stored at 4 °C. All samples were extracted in two runs.

Determination of the total phenolic content. The total phenolic content was determined according to a method developed by Gutfinger (1981). An appropriately diluted extract or caffeic acid (as calibration standard) was mixed with Folin-Ciocalteu reagent, sodium carbonate solution (20%, *w*/*v*) and Milli-Q water. After 40 min, absorbance was measured at 765 nm using a Hewlett Packard model 8453 UV-VIS spectrophotometer (Hewlett Packard, Waldbronn, Germany) with a 1-cm cell. The results were expressed in mg of caffeic acid (CA) per g of defatted seeds.

DPPH˙ radical scavenging activity. The effectiveness of the DPPH˙ radical scavenger was determined according to Brand-Williams et al. (1995). DPPH˙ solution (0.1 mmol, 3.9 mL) in 70% ethanol was added to 0.1 mL of the extract solution at various concentrations or to 0.1 mL of 70% ethanol (control). After 30 min, the absorbance was measured at 517 nm against 70% ethanol as a blank using a UV spectrophotometer (model 8453; Hewlett Packard, Waldbronn, Germany). The results were expressed as the phenolic concentration required to reduce the initial radical content by 50% (EC_{50}).

Cannabinoid content evaluation. Prior to cannabinoid determination, dry inflorescence samples collected at full flowering and seed maturation were prepared by removing the seed, large leaflets, supporting leaves and stems. Only flowers, bracts and small leaves (sugar leaves) of the inflorescence were collected. Air dried samples were ground to powder with liquid nitrogen (–196 °C), and 0.2 g of the sample was extracted with 10 mL of ethanol containing 10% chloroform (*v*/*v*) in a refrigerated ultrasonic bath at 0 °C for 30 min. The samples were centrifuged at 10 000 rpm for 10 min, filtered through 0.2-µm polyamide filters (Macherey-Nagel, Düren, Germany), transferred to a vial and stored at –20 °C until analysis by high-performance liquid chromatography (HPLC).

https://doi.org/10.17221/556/2025-PSE

The HPLC system on the Dionex Ultimate 3000 HPLC system (Thermo Scientific, Waltham, USA) was coupled to a diode array detector (Thermo Finnigan, San Jose, USA) at 272 nm and separation was performed on the Gemini C18 column (150 × 4.4 mm, 3 µm; Phenomenex, Torrance, USA) coupled to a Security Guard Cartridge (Gemini C18 × 3.0 mm). The chromatographic conditions were described by Stolker et al. (2004), with some modifications. The column was operated at 25 °C, and the temperature of the autosampler was maintained at 10 °C. The injection volume was 20 µL, and the sample analysis lasted 45 min at a flow rate of 0.6 mL/min. A gradient method was used in which mobile phase A consisted of 70% acetonitrile with 30% methanol and 0.1% formic acid (v:v:v) and mobile phase B consisted of 0.1% formic acid in double distilled water (B). The samples were eluted with a linear gradient of 40–20% B in the first minute, followed by an isocratic mixture for 21 min, a linear gradient of 20–5% B for 30 s, an isocratic mixture for 7 min, a linear gradient of 5–40% B for 30 s, an isocratic mixture for 6 min and a linear gradient of 40–100% B for 5 min, before returning to the initial conditions after 45 min.

Compounds were determined by their retention times, spectral data and fragmentation characteristics using a mass spectrometer (LTQ XL Linear Ion Trap Mass Spectrometer, Thermo Scientific, Waltham, USA) with electrospray ionisation (ESI) in positive (anthocyanins) ionization mode. Analysis was performed using a data-dependent MSn scan with a full scan from m/z 100–1 700. The capillary temperature was 320 °C, and the sheath gas and the auxiliary gas were 20 and 8 units, respectively. The source voltage was 0.1 kV. The spectrometric data were elaborated using Excalibur software (Thermo Scientific, San Jose, USA). The concentrations of each cannabinoid were calculated from the peak areas of the samples and the corresponding external standards obtained with external standard compounds (cannabidiol [CBD], cannabidiolic acid [CBDA], Δ9-tetrahydrocannabinol [Δ9-THC], Δ9-tetrahydrocannabinolic acid [Δ9-THCA], cannabinol [CBN]) and expressed as mg/g DW (dry weight). The conversion factor (0.877) for CBDA and THCA was used when the carboxylic acid derivatives (CBDA and THCA) were added with the non-carboxylic acid forms (CBD and THC) to the concentrations of total cannabinoids (total CBD [CBDt] and total THC [THCt]).

Statistical analysis. The agronomic performance variables studied over three years (seed yield, TSW, plant height and fresh stem yield) were first subjected to a combined analysis of variance (ANOVA). The year (growing season) was considered a random factor to normalise the influence of weather and to make general statements about the fixed factors, which were sowing density, variety and sowing density × variety interaction. The replicates were also considered random. For the analysis of the biochemical variables, which were investigated over two years (2017 and 2019), the growing season and the replicates were again considered as random factors, and a one-way ANOVA was performed for the variety as a fixed factor. The influence of variety on fatty acid composition was investigated and analysed only for the 2017 growing season, considering variety as a fixed factor. For the analysis of cannabinoid content in 2019, a two-factorial ANOVA was performed with variety, time of sampling and the variety × time of sampling interaction as fixed factors. Before analysis, each response variable was tested for the assumptions of a normal distribution and homogeneity of treatment variances using the Levene test. In the case of non-homogeneity of variances, the data were transformed to log(y) or sqrt(y). Analysis of variance and correlation analysis were performed with the packages 'agricolae' and 'nlme' in the statistical software program R version 3.2.5 (R Core Team 2019, http://www.r-project.org). Significant differences in means indicated by ANOVA were assessed using Duncan's test (α = 0.05). The data are presented as untransformed means ± standard error (SE). The graphs were created using the Microsoft Excel program (San Jose, USA).

RESULTS AND DISCUSSION

Agronomic performance. Most examined agronomic variables differed significantly among the hemp varieties tested in this study (Table 2), but no statistical differences were found in plant height, with the tallest variety being Tiborszallasi (141 cm) and the lowest USO 31, Fedora 17 and Santhica 27 (≈ 109 cm). The TSW varied between 16.4 g (Tiborszallasi) and 12.4 g (Santhica 27 and USO 31). Correlation analysis showed that seed yield was not correlated with TSW, but a high and statistically significant (P < 0.001) correlation was observed with plant height (0.70) and fresh stem yield (0.77). A high (0.81) and statistically significant (P < 0.001) correlation was also found between plant height and fresh stem yield (Figure 3).

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Table 2. Seed yield, 1 000-seed weight (TSW), plant height and fresh stem yield of 7 hemp varieties from 3-year field experiments

Variety	Seed yield (kg/ha)	TSW (g)	Plant height (cm)	Fresh stem yield (kg/ha)
Futura 75	505 ± 96^{a}	16.3 ± 0.5^{a}	137.8 ± 11.6	8 036 ± 1 232^{a}
Tiborszallasi	372 ± 69^{ab}	16.4 ± 0.6^{a}	140.6 ± 13.1	7 682 ± 1 171^{ab}
KC Dora	320 ± 53^{b}	15.1 ± 0.4^{ab}	136.4 ± 11.6	7 235 ± 1 275^{ab}
Fedora 17	290 ± 46^{b}	13.9 ± 0.4^{b}	109.6 ± 10.3	4 830 ± 792^{abc}
Monoica	286 ± 56^{b}	15.7 ± 0.4^{a}	131.9 ± 13.8	6 653 ± 1 411^{ab}
Santhica 27	198 ± 53^{b}	12.4 ± 0.3^{c}	108.2 ± 10.7	4 253 ± 899^{bc}
USO 31	186 ± 37^{b}	12.4 ± 0.5^{c}	109.7 ± 9.9	2 904 ± 440^{c}
P	**	***	ns	**

***P < 0.001; **P < 0.01; *P < 0.05; ns – not significant; different lowercase letters within a column indicate significant differences between values, according to Duncan's test ($P \leq 0.05$). Overlapping letters indicate non-significant differences

The yields of hemp stem and seed can vary considerably, and the scientific literature contains very different data. For example, Tang et al. (2016) determined up to 2.4 t/ha seed yield in France (variety Fedora 17) and up to 22.1 t/ha dry stem yield in Latvia (variety KC Dora); Baldini et al. (2020) measured a maximum of 1 t/ha of seed and up to 12 t/ha of dry stalks in an experiment with six European hemp varieties in northeast Italy. Ferfuia et al. (2021) evaluated five monoecious varieties across six environments in northern Italy and reported yields of up to 1.2 t/ha of seed and up to 7 t/ha of dry stalks. A study in northern Greece has shown seed yields of up to 2.9 t/ha and stem yields of up to 18.6 t/ha (Tsaliki et al. 2021). In our study, the yields were relatively low. Futura 75 stood out with more than 0.5 t/ha of seed and over 8 t/ha of fresh stems; otherwise, the seed yields were below 0.4 t/ha, and the average stem yields of the

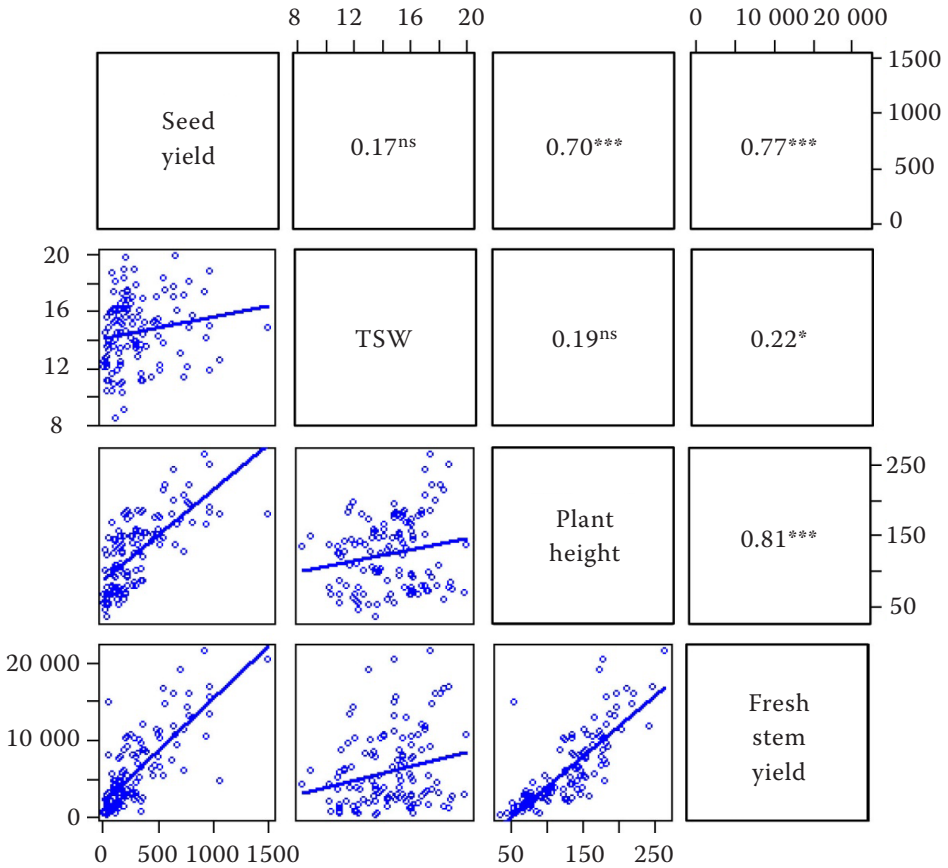

Figure 3. Correlation plots among seed yield, TSW (1 000-seed weight), plant height and fresh stem yield; ***P < 0.001; *P < 0.05, ns – not significant

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other varieties were below 5.6 t/ha. Stem yield was strongly influenced by variety, with the best result at Futura 75 (8.0 t/ha) having a 2.8-fold higher fresh stem yield than USO 31 (2.9 t/ha). In the variety trial at the same location in the previous study, the highest seed yield was recorded for the variety Futura 75 (seed yield 1.57 t/ha, stem yield 3.2 t dry matter/ha) as well (Flajšman et al. 2017). However, although the weather conditions in 2016 at the Ljubljana location were relatively favourable for hemp growth, stem yields were low compared to similar field trials elsewhere (Flajšman et al. 2017), indicating the combination of weather conditions and soil type (heavy soil) that prevail at this location is not conducive to hemp stem production. The stem yield of this variety was reported to range between 5 and 13 t/ha in trials in Italy (Amaducci et al. 2008, Cosentino et al. 2012), 11 t/ha in Greece (Papastylianou et al. 2018), around 10 t/ha in Sweden (Svennerstedt and Sevenson 2006) and in France (Tang et al. 2016, Harrabi et al. 2017), 15.2 t/ha in the Czech Republic, and up to 20 t/ha in Latvia (Ivanovs et al. 2015, Tang et al. 2016). The highest seed yield for this variety was recorded in trials in Italy, reaching 1.4 t/ha, while yields in trials in the Czech Republic were 1 t/ha and in France 0.5 t/ha (Tang et al. 2016). In Italy, lower seed yields were also recorded, namely 0.4 t/ha (Baldini et al. 2018) and 0.15 t/ha (Campiglia et al. 2017). In a field trial conducted in Ljubljana in 2016 at the same location as the presented investigation, Futura 75 proved to be the most productive variety among the seven studied, with a stem yield of 3.3 t/ha and a seed yield of 1.6 t/ha (Flajšman et al. 2016).

The goal of hemp breeding to increase seed yield has been partially achieved through the development of monoecious varieties, which generally have a higher seed yield than dioecious varieties (Berenji et al. 2013). In our experiment, the highest 3-year average seed yield was obtained with the monoecious variety Futura 75 (505 kg/ha), followed by the diecious variety Tiborszallasi (372 kg/ha). The lowest yields were recorded for monoecious varieties Santhica 27 (198 kg/ha) and USO 31 (186 kg/ha). Related to these seed yield results, hemp remains uncompetitive compared to other crops, e.g., cereals, and the search for optimal cultivation practices for seed production across different environments and for other uses, especially for its quality, is even more important for profitable production.

Hemp cultivation is highly dependent on weather conditions (Baldini et al. 2020, Ferfuia et al. 2021). Tsaliki et al. (2021) showed a significant effect of growing year on yield, especially on stem yield. Regarding seed yield, Baldini et al. (2020) clearly showed that excessive temperatures and a lack of rainfall during the grain-filling period led to a significant reduction in seed production. Similarly, Ferfuia et al. (2021) confirmed that among climate parameters, high temperatures during the early grain-filling period led to a progressive decrease in seed yield. There have been further reports of large differences in seed and stem yields of the same variety in different growing years (e.g. Tang et al. 2016), confirmed also with results of our study. At the same time, in our study, stem and seed yield were highly and significantly correlated ($R = 0.77$, $P < 0.001$), indicating that weather strongly similarly influenced both. The first year of the field trial had the most favourable conditions for hemp growth; the highest seed and stem yields in that year were 0.93 t/ha dry matter and 12.3 t/ha fresh matter, respectively, for the Futura 75 variety, and 0.447 t/ha and 7.768 t/ha, respectively, in the average of all varieties. Compared to the first year, the seed and stem yields were lower by 29.3% and 3.2%, respectively, in the second year and by 63.8% and 67.4%, respectively, in the third year. The extensive rainfall after sowing in May 2018 on the heavy soil in our experiment likely reduced emergence and increased weed competition, as supported by Baldini et al. (2020), who highlighted the adverse impact of excessive moisture during establishment. Poor emergence due to excessive rainfall in May 2019, accompanied by hot, dry conditions during grain filling, which likely caused seed abortion and reduced filling, as confirmed by Ferfuia et al. (2021), further exacerbated the negative impact on seed yield. Futura 75 consistently outperformed other varieties, even in stress years; however, seed yield still declined sharply in this variety under such conditions (934 kg/ha, 280 kg/ha, and 302 kg/ha in 2017, 2018, and 2019, respectively).

Sowing density (SD) is one of the three most important factors influencing the final yield of hemp seeds and biomass, along with nitrogen fertilisation and harvest time (Poniatowska et al. 2022), and one of the most studied parameters of hemp cultivation (Amaducci et al. 2015). Several studies have confirmed the significant effect of SD on yield (Yazici 2023), however in our study, no effect of SD was observed on hemp seed yield or other agronomic parameters. ANOVA showed that only variety had a statistically significant effect on the agronomic performance,

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while SD and the SD × variety interaction had no effect. The difference of 50 plants/m^2 between SD1 (200 seeds/m^2) and SD2 (150 seeds/m^2) in our study was obviously too small to have a statistically significant impact in the measured variables.

To conclude, in a 3-year field trial in Slovenia (humid continental climate with oceanic influences, heavy soil) with seven EU hemp varieties Futura 75 and Tiborszallasi showed the best results, although yields were generally low. Futura 75 achieved the highest seed yield (505 kg/ha dry matter), stem yield (8 036 kg/ha fresh matter), protein yield (140 kg/ha) and oil yield (181 kg/ha). Seed and stem yields declined significantly under unfavourable emergence conditions (excessive rainfall after sowing) and heat/drought stress during grain filling. Stem and seed yields were strongly correlated, indicating that weather affected both vegetative and reproductive growth in a similar way. These findings provide insights relevant to regions with similar temperate climates. Identifying varieties with superior performance in comparable environments provides practical guidance for growers. It underscores the importance of targeted variety selection and the need for research in the field of management strategy optimisation.

Oil content and oil yield. The variety factor had a statistically significant influence on both the seed oil content ($P < 0.01$) and oil yield ($P < 0.01$) (Figure 3). Santhica 27 had the lowest oil content at 23.1%, followed by USO 31; the highest value was recorded for Tiborszallasi (30.2%) (Figure 4).

Some of the investigated hemp varieties showed lower oil content than reported in the literature; for example, Tiborszallasi reached 30.2%, compared to 37.8% (Schultz et al. 2020), 34.8% (Galasso et al. 2016), and 30.6% (Vonapartis et al. 2015). Alonso-Esteban et al. (2022) reported a similar oil content of the Tiborszallasi variety (29.1%). Four of the varieties used in the field trials by Schultz et al. (2020) and Galasso et al. (2016) were also analysed in our study. KC Dora and USO 31 had much lower oil content in the current study (28.1% and 23.6%, respectively) than in Schultz et al. (2020), where oil contents of 32.8% and 37.2%, respectively, were detected. In addition, KC Dora reached 31.5% in Galasso et al. (2016).

In contrast, Futura 75 and Fedora had 29.8% and 27.3% oil content, respectively, with little difference compared to the results of Galasso et al. (2016) (29.8% and 29.9% for Futura 75 and Fedora, respectively). The oil content of hemp seeds is influenced by varietal differences; however, other factors, such as environmental conditions, seed maturity, and processing methods, can also affect oil content. Irakli et al. (2019) showed that protein and oil content, as well as fatty acid composition, were primarily influenced by genetic factors. In contrast, total phenolic, tocopherol, and carotenoid content, and antioxidant activity were mainly influenced by year of cultivation and genotype.

Of all the varieties studied, only Futura 75, with a yield of 181 kg/ha, showed a statistically significant difference in oil yield (Figure 4). The average oil yield was highest for Tiborszallasi, Futura 75, Monoica,

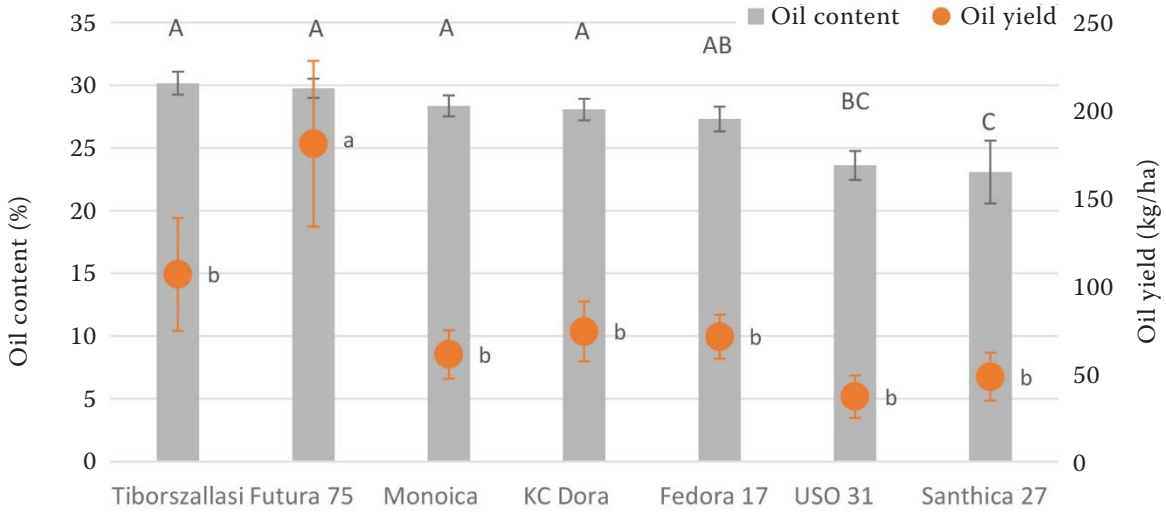

Figure 4. Oil content and yield of hemp varieties. Different capital letters indicate statistically significant differences in oil content between varieties. Overlapping letters indicate non-significant differences ($P \leq 0.05$, Duncan's test). Different lowercase letters indicate statistically significant differences in oil yield between the varieties ($P \leq 0.05$, Duncan's test)

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and KC Dora, at 106 kg/ha, compared to USO31 and Santhica 27, which had the lowest oil yield at 43 kg/ha. We can see that oil yield is influenced much more by seed yield than by seed oil content.

To conclude, the results confirm that varietal differences significantly influenced both hemp seed oil content and oil yield. However, environmental conditions likely contributed to lower oil percentages than those reported in the literature. Oil yield was more strongly determined by seed yield than by oil content.

Protein content and protein yield. Variety had no significant effect on protein content, which was lower than in other studies, ranging from 19.6% to 21.8% (Figure 5). Vonapartis et al. (2015) conducted a field study with 10 hemp varieties in Canada and reported protein content of 23.8–28.0%. In an Italian study with 20 hemp varieties and wild hemp accessions, Galasso et al. (2016) reported protein content of 31.6–35.6% in dry matter. An analysis of the composition of 7 hemp varieties grown in Greece over 3 years showed that protein content ranged from 12.2% to 25.4% (Irakli et al. 2019), consistent with our results.

However, variety had a significant effect on protein yield ($P < 0.01$), mainly due to differences in seed yield. It was significantly higher in the Futura 75 (140 kg/ha), followed by the Tiborszallasi (79 kg/ha), compared to all other varieties tested (32–58 kg/ha) (Figure 5).

To conclude, Futura 75 was particularly notable under the experimental conditions, achieving the highest seed yield (505 kg/ha), fresh stem yield (8 036 kg/ha), oil yield (181 kg/ha), and protein yield (140 kg/ha). Based on the results of this study, Futura 75 was selected as one of the parent varieties for use in the breeding program at the Biotechnical Faculty to breed Slovenian monoecious varieties.

Fatty acid composition. Statistically significant differences were observed among the varieties for all fatty acids measured in this study. Among the unsaturated acids, linoleic acid (omega-6) is the most represented (42.7–53.9%), followed by oleic acid (omega-9) (13.2–18.4%) and α-linolenic acid (omega-3) (9.3–18.2%). The aforementioned unsaturated fatty acids play a protective role in preventing cardiovascular disease, which is why it is recommended that they make up a large proportion of the fats consumed with food (Mišurcová et al. 2011). The total unsaturated fat content in the seeds ranged from 71.8% (Santhica 27) to 87.6% (Tiborszallasi). The highest content of linoleic and α-linolenic acid was found in varieties Tiborszallasi (53.8%; 18.2%), Futura 75 (52.8%; 16.3%) and KC Dora (52.2%; 16.2%), and oleic acid in USO 31 (18.4%) (Table 3). Variability in unsaturated fatty acid content was also observed in similar studies investigating the influence of genotype on fatty acid composition (Galasso et al. 2016).

The ratio of omega-6 to omega-3 fatty acids also plays an important role and is already more favourable in hemp oil than in other vegetable oils, such as sunflower, corn, and others (Dulf et al. 2006). The current Western diet is deficient in food sources with an optimal ratio of linoleic and α-linolenic acids (between 3:1 and 5:1; EFSA 2009) generally with a high (> 15:1) ratio of omega-6:omega-3, which is due to the increased consumption of vegetable oils,

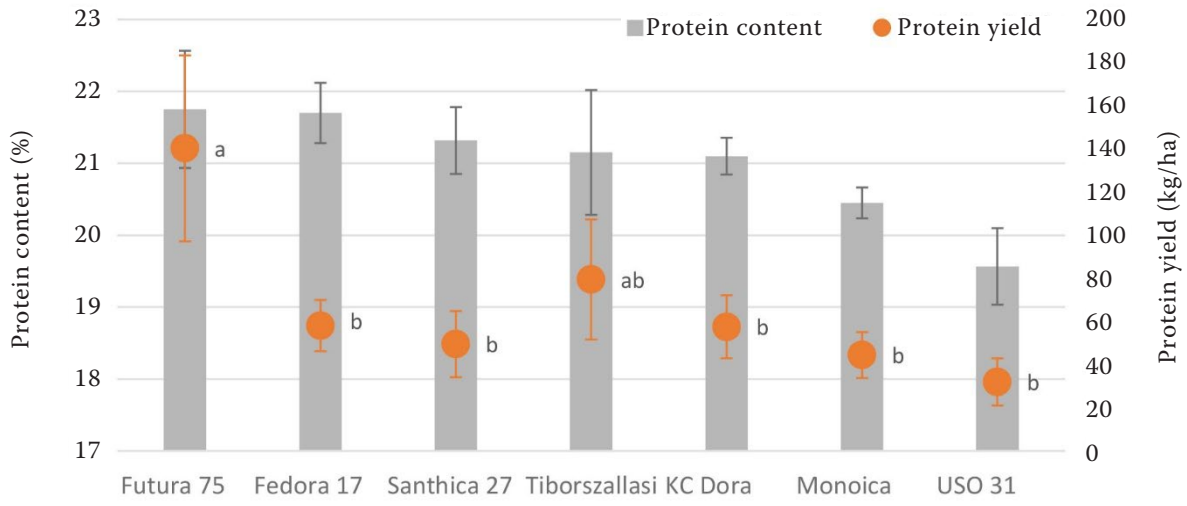

Figure 5. Protein content and yield of hemp varieties. Different lowercase letters indicate statistically significant differences in protein yield between the varieties. Overlapping letters indicate non-significant differences ($P \leq 0.05$, Duncan's test). There was no significant influence of the variety on the protein content

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Table 3. The content (the unit is per cent) of 10 fatty acids in hemp seeds is influenced by the variety

Variety	PA16:0	PAL16:1	SA18:0	OA18:1*cis*9	LA18:2&6	GLA18:3&6	ALA18:3&3	ARA20:0	GOA20:1cis11	BA22:0	UFA	Ω3	Ω6	Ω6:Ω3
Santhica 27	12.62^{a}	0.20^{a}	4.95^{a}	17.0^{ab}	42.7^{b}	1.11^{cd}	9.3^{c}	0.08^{a}	0.67^{a}	0.84^{a}	71.8^{c}	9.9^{c}	43.9^{b}	4.6^{a}
USO 31	11.02^{ab}	0.18^{ab}	4.63^{ab}	18.4^{a}	42.3^{b}	0.97^{d}	11.1^{c}	0.08^{a}	0.62^{ab}	0.75^{ab}	74.1^{bc}	11.6^{c}	43.2^{b}	4.2^{ab}
Fedora 17	9.27^{bc}	0.15^{bc}	3.65^{bc}	15.1^{abc}	49.5^{a}	1.73^{a}	12.5^{bc}	0.08^{a}	0.54^{abc}	0.61^{bc}	80.3^{ab}	13.1^{bc}	51.2^{a}	4.0^{ab}
Monoica	8.72^{bc}	0.15^{bc}	3.78^{bc}	15.9^{abc}	49.9^{a}	1.02^{cd}	13.6^{abc}	0.08^{a}	0.45^{c}	0.51^{c}	81.9^{ab}	14.3^{abc}	51.0^{a}	3.7^{abc}
KC Dora	7.99^{c}	0.14^{c}	3.53^{c}	13.5^{bc}	52.2^{a}	1.34^{bc}	16.2^{ab}	0.05^{b}	0.49^{bc}	0.51^{c}	84.3^{a}	16.6^{ab}	53.6^{a}	3.2^{bc}
Futura 75	7.21^{c}	0.13^{c}	3.36^{c}	14.0^{bc}	52.7^{a}	1.55^{ab}	16.3^{ab}	0.05^{b}	0.44^{c}	0.46^{c}	85.5^{a}	16.5^{ab}	54.4^{a}	3.2^{bc}
Tiborszallasi	6.69^{c}	0.12^{c}	2.91^{c}	13.2^{c}	53.9^{a}	1.75^{a}	18.2^{a}	0.02^{c}	0.43^{c}	0.41^{c}	87.6^{a}	18.2^{a}	55.7^{a}	3.0^{c}
P	**	**	**	*	**	***	*	***	**	**	**	*	**	*

PA – palmitic acid; PAL – palmitoleic acid; SA – stearic acid; OA – oleic acid; LA – linoleic acid; GLA-γ – linolenic acid; ALA-α – linolenic acid; ARA – arachidic acid; GOA – gondoic acid; BA – behenic acid; UFA – unsaturated fatty acids; $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ns – not significant; different lowercase letters within a column indicate significant differences between values, according to Duncan's test ($P \leq 0.05$). Overlapping letters indicate non-significant differences

including sunflower and corn oil, which have a ratio of > 50:1 omega-6:omega-3 (Schultz et al. 2020). The results of the present analysis showed that the fatty acid composition of the produced hemp oil falls into the high linoleic acid and low α-linolenic acid category, with a ratio of 4.6:1 to 3.0:1, which is close to the recommended ratio (EFSA 2009).

Some studies have suggested that stress can reduce the oil or protein content and seed yield and alter fatty acid profiles (Irakli et al. 2019).

To conclude, related to the ratio of omega-6:omega-3, varieties Tiborszallasi (3.0:1), Futura 75 (3.2:1) and KC Dora (3.2:1) clearly stood out.

Total phenolic content and DPPH˙ radical scavenging activity. Statistical analysis showed that the total phenolic content ($P < 0.01$) was significantly influenced by the variety (Figure 6). The values expressed as CA equivalents per gram of defatted seeds are averages over 2 years. The parameter was measured on whole seeds (i.e., the seeds were not hulled before homogenisation and defatting). The highest total phenolic content was found in the seeds of the Tiborszallasi variety, and the lowest in the seeds of the USO 31 variety. The total phenol content in the seeds of the varieties Monoica, KC Dora, Futura 75, and Fedora 17 did not differ significantly. Comparable values were obtained by Frassinetti et al. (2018), who determined the total phenolic content in extracts prepared with 80% ethanol from hemp seeds of the Futura 75 variety. On the other hand, Occhiuto et al. (2022) in their investigation of defatted hempseed meal from the USO 31 and Futura 75 varieties found significantly higher total phenolic content in the former. Galasso et al. (2016) determined the total phenolic content in defatted seeds of 20 industrial hemp varieties and found statistically significant differences among the varieties, with values ranging from 5.9 to 10.6 mg CA/g. Higher values than in our study should be attributed, among other things, to the higher extraction temperature (70 °C), which yielded a higher extraction yield than in our experiment, conducted at room temperature. Teh et al. (2014) investigated the phenolic content of ethanolic extracts from the defatted meal of industrial hemp seeds. Their values of about 3.5 mg GA/g, expressed as gallic acid equivalents (GA), are slightly higher than the values we determined. Taaifi et al. (2021) reported values of 1.3–2.0 mg GA per g of hemp seed, depending on the production region, which are comparable to ours, assuming a fat content of about 30% in the seeds. However, the results should not be generalised, as in

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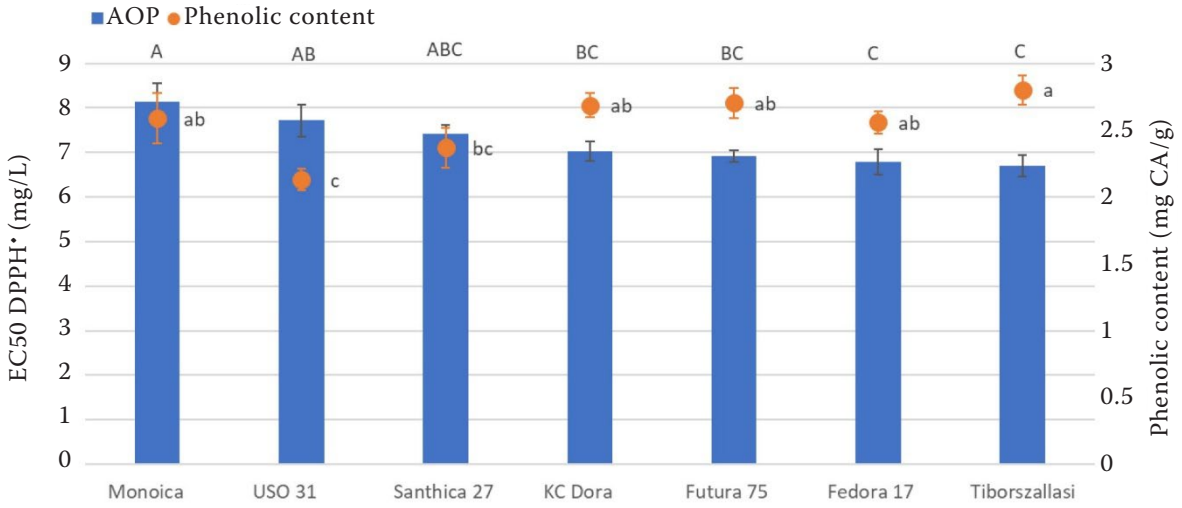

Figure 6. The total phenolic content and DPPH˙ (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity (AOP – antioxidant potential) of the hemp varieties tested. Different lowercase letters indicate statistically significant differences in total phenolic content among the varieties. Overlapping letters indicate non-significant differences ($P \leq 0.05$, Duncan's test). Different capital letters indicate statistically significant differences in antioxidant potential between the varieties. Overlapping letters indicate non-significant differences ($P \leq 0.05$, Duncan's test)

the study mentioned, the determination was carried out in a 90% methanolic extract. The influence of the extraction solvent on the quantity and composition of the phenols is known (Martinez et al. 2020). Teh et al. (2014) determined an almost 25% higher total phenol content in methanolic extracts from hemp seeds than in ethanolic extracts.

As reviewed recently by Hossain et al. (2025), different studies have confirmed the effectiveness of ethanolic extracts from hemp seeds as free radical scavengers and emphasised that the total phenol content and composition of individual phenols in the extract strongly depended on the growing conditions, the extraction method, the calibration standard compound used and the presentation of the data. Therefore, it is hardly possible to compare values across studies. Irakli et al. (2019) found that total phenol levels were more strongly influenced by year of cultivation than by genotype, with a trend of higher total phenol levels in years with lower rainfall.

Furthermore, researchers (Faugno et al. 2019) observed that the production of phenolic compounds was maximised when seeds were harvested at high density (60 plants/m^2) in soils that did not undergo pre-fertilisation. On the other hand, Przybylska-Balcerek et al. (2024) did not confirm that the cultivation method affects the phenolic content.

Variety also had a statistically significant influence ($P < 0.01$) on the antioxidant potential (AOP) (Figure 6). Samples with a lower EC_{50} (concentration required to achieve a 50% antioxidant effect) had a better AOP. Of the ethanolic extracts obtained from defatted hemp seeds, Tiborszallasi and Fedora 17 were the most effective free radical scavengers. At the same time, Monoica, with a moderate total phenolic content, had the lowest AOP (Figure 6). Except for Fedora 17 and Monoica, the order of EC_{50} followed the order of total phenolic content (Pearson correlation coefficient = –0.988).

Chen et al. (2012) performed DPPH analysis with 75% ethanolic extracts obtained from dehulled hemp seeds of 2 varieties. Considering their EC_{50} values, which ranged from 10 to 20 mg GA/L, the AOP varied by cultivar and was lower than that of the hemp seeds in our study. However, in the same study, a significantly higher AOP was measured for the extract from hemp seed hulls, which showed a large proportion of polyphenols. Occhiuto et al. (2022) also confirmed the variety dependence of AOP, whereby the extract from USO 31 was more powerful than that from Futura 75, which contrasts with our observations.

Our ethanolic extracts showed a significantly higher AOP than butylated hydroxytoluene (BHT) (EC_{50} = 13.6 ± 0.2 mg/L). The synergistic effect of structurally distinct antioxidants in the extracts on free radical degradation was consistently observed. Previous studies have shown that among the phenolic compounds in hemp seeds, flavonoids, especially flavanones, flavonols, flavanols and isoflavones, are the most

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abundant (Occhiuto et al. 2022). Phenolic acids, such as *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, sinapic, ferulic, syringic, gallic and benzoic, are also present (Martinez et al. 2020, Occhiuto et al. 2022, Przybylska-Balcerek et al. 2024). Quercetin and luteolin, followed by caffeic acid, were identified by Teh et al. (2014) as the most important polyphenols in ethanolic extracts from hemp seeds, which are known to be good free radical scavengers. Some representatives of stilbenes and lignans, in particular lignanamides (also called cannabisines), are also present in hemp seeds (Martinez et al. 2020). The primary lignanamides identified in hemp seeds are *N-trans*-feruoyltyramine or *N-trans*-caffeoyltyramine, which, in addition to their antioxidant effect, also have important anti-neuroinflammatory activities (Martinez et al. 2020).

To conclude, among the tested varieties, Tiborszallasi had the highest total phenolic content and was the most effective free radical scavenger. The order of AOP is generally followed by the order of total phenolic content.

Cannabinoid content. A significant interaction effect between sampling time and variety was observed for the contents of CBDt (Figure 7), THCt (Figure 8), CBD, CBDA, THCA and CBN (data not shown). In contrast, for THC and the CBDt: THCt ratio (data not shown), only the variety was significant. The highest CBDt content was measured at seed harvest for Tiborszallasi (4.3%), Futura 75 (3.9%), KC Dora (3.8%) and Monoica (3.5%) (Figure 7). In general, the average CBDt content was 2.1% at flowering and 2.6% at seed harvest.

The highest THCt content was measured at seed harvest in Tiborszallasi (0.51%) and Monoica (0.33%). At flowering, 0.32% was measured in Tiborszallasi, followed by KC Dora at seed harvest (0.22%) and at flowering (0.21%). All other combinations of sampling time and variety contained less than 0.20% THCt. USO 31 at seed harvest and Santhica 27 at both sampling times contained THCt below the detection limit (Figure 8). The average THCt content was 0.16% at flowering and was 0.27% at seed harvest. Some samples exceeded the EU limit of 0.3% for Δ9-THC (Regulation (EC) No 2021/2115), raising concerns. However, various agrotechnological measures can significantly influence the composition of cannabinoids. For example, higher seeding density and fertiliser omission increased the CBD and d9-THC content of the Felina 32 variety in the northern Baltic climate (Barčauskaitė et al. 2022). CBD production of CBD varieties also increased with increasing plant density in northern Florida (USA), but mainly due to the higher flower yield (da Silva Benevenute et al. 2022). In contrast, Poniatowska et al. (2022) found no effect of sowing density (60 and 180 seeds/m^2) on CBD yield. Other factors that can influence cannabinoid content in hemp include temperature, sex ratio, day length, plant maturity, irrigation, soil chemical composition and nutrient availability, and ultraviolet light intensity (Barčauskaitė et al. 2022). Significant variations in cannabinoid content were observed both among the fifteen varieties studied, grown under uniform conditions (ANOVA, $P < 0.05$) and within individual varieties, indicating heterogeneous genetic profiles of the seed material.

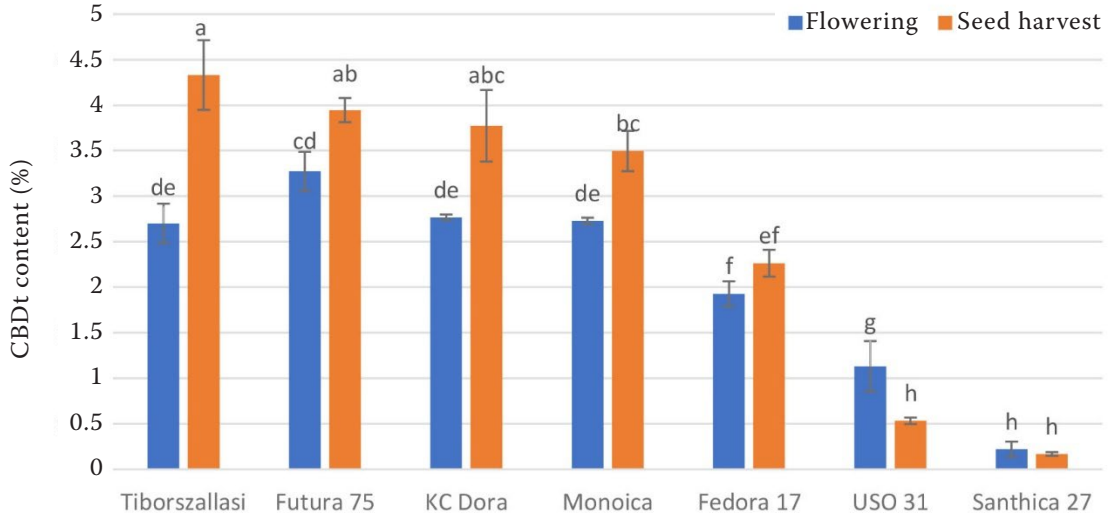

Figure 7. Interaction effect of sampling time × variety ($P < 0.001$) on the total cannabinoids (CBDt) content; different lowercase letters above the bars indicate significant differences between the means according to Duncan's test ($P \leq 0.05$). Overlapping letters indicate non-significant differences

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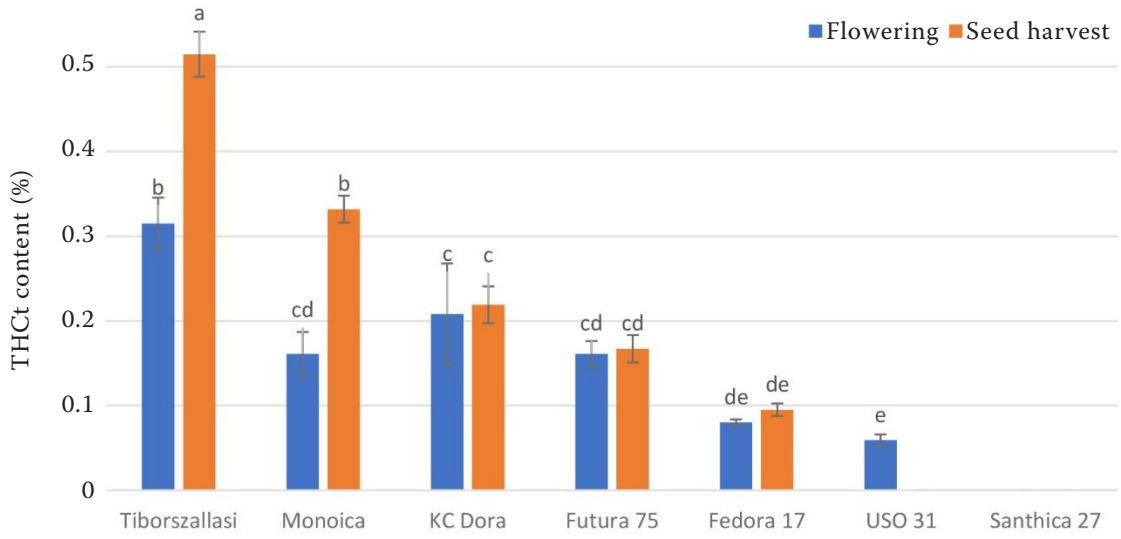

Figure 8. Interaction effect of sampling time × variety ($P < 0.01$) on the total tetrahydrocannabinol (THCt) content; different lowercase letters above the bars indicate significant differences between the means according to Duncan's test ($P \leq 0.05$). Overlapping letters indicate non-significant differences

Among the tested varieties, Fedora 17, USO 31, Tisza, Tiborszallasi, and Antal demonstrated a favourable response to the growing conditions in Slovenia, as indicated by the experiment by Glivar et al. (2020) on cannabinoid accumulation. A total of 13 cannabinoids were determined. A tetrahydrocannabinol/cannabidiol ratio > 1:30 was found in four cultivars in their experiment.

Pollination of cannabis flowers has been shown to reduce the CBD and THC contents, which also decrease with plant age (Todd et al. 2022). Our study showed the opposite result: an increase in CBDt and THCt content when sampled at seed maturity compared to full flowering. Our results are consistent with those of Calzolari et al. (2017) and others, who also conducted field studies and found that total CBD and THC levels were higher at plant maturity than at flowering. One reason for this result could be that longer growing seasons lead to higher GDDs, which are positively correlated with CBD and THC accumulation in flower extracts (Sikora et al. 2011). In contrast, Todd et al. (2022) found that mature Finola plants with induced seeds had a statistically significantly lower CBDA content and a total CBD content that was 2 times lower than that of unpollinated plants. Yield of inflorescences at seed harvest was not evaluated in our study; therefore, CBD production could not be assessed. However, the higher cannabinoid content at seed harvest in the majority of the tested varieties represents the opportunity to use them as dual-purpose varieties, namely for seed production and flower production for cannabinoid extraction.

The ratio between the two main cannabinoids (CBD and THC) is very important for hemp growers to ensure compliance with regulations with low THC levels while maximising CBD yields. Therefore, a high CBD content and a broad CBDt:THCt ratio are desirable when hemp is grown outdoors and used for cannabinoid production.

The CBD:THC ratio defines the chemotype of cannabis plants and is genetically determined (Toth et al. 2021). However, various environmental and growth stressors (e.g., agronomic practices, soil conditions, and biotic and abiotic factors) can alter cannabinoid content and the CBD:THC ratio (Campbell et al. 2019). Calzolari et al. (2017), for example, found that the CBD/THC ratio increased when harvest was delayed after seed maturity. However, Toth et al. (2021) found no effects of five different stress factors (plant growth regulator ethephon, flooding, powdery mildew, herbicide and physical wounding) on the ratio of total CBD to total THC at harvest. These observations are consistent with our results, which showed that sampling timing did not affect the CBD:THC ratio. However, there were statistically significant differences among varieties, with the CBDt:THCt ratio ranging from 8.6 for Tiborszallasi to 24.0 for Fedora 17 (data not shown). This result was expected, and a significant influence of variety on the CBD:THC ratio has also been shown in previous studies (Campbell et al. 2019).

To conclude, among the tested varieties, Futura 75 and Tiborszallasi exhibited not only the best agronomic

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performance, but also the highest oil content, the highest omega-6 fatty acid level, the most favourable omega-6 to omega-3 ratio, the highest total phenolic content, superior antioxidant potential, and the highest CBDt content in the inflorescences at flowering and seed harvest. Tiborszallasi exhibited the most favourable $\omega 6{:}\omega 3$ fatty acid ratio in the seeds (3:1), the highest oil content (30.2%), and the greatest total phenolic content (2.8 mg CA/g). It also demonstrated the strongest antioxidant potential (EC_{50} DPPH: 6.69 mg/L) and a high total unsaturated fatty acid content (87.6%).

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