

From breeding to practise: Effects of sowing date and pruning system on flower yield and cannabinoid composition in four Slovenian feminized industrial hemp breeding lines

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Received February 05, 2026, accepted February 13, 2026
Delo je prispelo 5. februar 2026, sprejeto 13. februar 2026

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Abstract: Four Slovenian feminized industrial hemp (*Cannabis sativa* L.) breeding lines (G8c, G10c, MERc, CHc), developed within the high-CBD breeding program at the Biotechnical Faculty, University of Ljubljana, were evaluated in 2024 field trials at Ljubljana and Krško. The effects of sowing date (direct field seed sowing vs. seedlings transplantation to the field, both at density 8,333 plants per ha) and apical pruning (topping) on growth (plant height), flower yield, CBD, and Δ^9 THC content were assessed. Early-season waterlogging in Ljubljana (heavy soil) caused severe negative effects on plant establishment and growth. Sowing date acted oppositely across sites: direct sowing outperformed transplanting in Krško (medium heavy soil), whereas transplanting increased flower yield in Ljubljana. Pruning reduced plant height but did not change flower yield or cannabinoid concentrations. CBD was primarily genotype dependent, with MERc the highest at both sites (5.02–6.66 %). THC ranged 0.12–0.21 % across treatments. Significant breeding line $\times$ sowing date interactions occurred for plant height and flower yield. This study provides the first applied evaluation of Slovenian feminized industrial hemp breeding lines and indicates priorities for subsequent breeding efforts. However, the results represent one year of field data. To draw robust conclusions, multi-year field trials will be required.

Key words: industrial hemp, feminized hemp, high-CBD genotype, sowing date, transplanting, pruning, CBD, THC

Od žlahtnjenja do uporabe: Vpliv datuma setve in rezi na pridelek cvetov in vsebnost kanabinoidov v štirih slovenskih žlahtnjenih linijah feminizirane industrijske konoplje

Izveček: Štiri slovenske žlahtnjene linije feminizirane industrijske konoplje (*Cannabis sativa* L.) (G8c, G10c, MERc, CHc), razvite v okviru žlahtnjenja konoplje z veliko vsebnostjo CBD na Biotehniški fakulteti Univerze v Ljubljani, so bile ocenjene v poljskih poskusih leta 2024 v Ljubljani in Krškem. Ocenjeni so bili učinki datuma setve (neposredna setev semen na njivo in sajenje sadik: gostota 8333 rastlin na ha) in apikalne rezi na višino rastlin, pridelek cvetov, vsebnost CBD in Δ^9 THC. V Ljubljani je zastajanje padavin na tleh (težka tla) močno omejilo rast in razvoj rastlin. Način setve je imel različne vplive na pridelek cvetov: v Krškem (srednje težka tla) je neposredna setev pomenila večji pridelek socvetij, medtem ko je v Ljubljani presajanje sadik povečalo pridelek. Rez je zmanjšala višino rastlin, vendar ni vplivala na pridelek cvetov niti na koncentracije kanabinoidov. Vsebnost CBD je bila pretežno odvisna od linije; linija MERc je imela največjo vsebnost CBD na obeh lokacijah (5,02–6,66 %). THC je znašal 0,12–0,21 % v vseh obravnavanjih. Značilne so bile interakcije žlahtnitelske linije $\times$ način setve za višino rastlin in pridelek cvetov. Ta študija predstavlja prvo ocenjeno realnega pridelovalnega potenciala slovenskih žlahtnjenih linij feminizirane industrijske konoplje in nakazuje prednostne naloge za nadaljnje žlahtnjenje.

Ključne besede: industrijska konoplja, feminizirana konoplja, CBD konoplja, datum setve, presajanje, rez, CBD, THC

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1 INTRODUCTION

Industrial hemp (*Cannabis sativa* L.) has re-emerged as an important multipurpose crop in Europe and globally, driven by increasing demand for sustainable raw materials, plant-based bioactive compounds, and high-value cannabinoids. Although hemp has historically been cultivated primarily for fibre and seed, the past decade has witnessed rapid expansion of production systems focused on floral biomass rich in non-psychoactive cannabinoids, particularly cannabidiol (CBD) (Andre et al., 2016; Rehman et al., 2021; Pancaldi et al., 2025). This shift has renewed interest in agronomic practices that optimise inflorescence yield and cannabinoid composition, especially in high-CBD hemp varieties developed specifically for inflorescence production. As cultivation intensifies, understanding how production techniques influence both biomass productivity and phytochemical profiles has become essential for breeders, growers, and processors (Sebastian et al., 2023).

In recent years, cultivation of hemp for cannabinoids has become widespread due to the supported health benefits of cannabinoids. Consequently, a new category of industrial hemp varieties—so-called high-CBD varieties—has been introduced. Beyond elevated CBD content, these cultivars are feminised, meaning that each seed produces a female plant (Flajšman et al., 2021). The first high-CBD varieties were registered in the EU Plant Variety Database in 2022 (Plant Variety Database, 2026) and were cultivated in the 2023 season. Several field evaluations of foreign feminised hemp varieties for cannabinoid production were conducted in Slovenia (Korošec, 2024). The Biotechnical Faculty, University of Ljubljana, also performed field trials with feminised hemp (unpublished results). Across trials, foreign varieties generally ripened very late under Slovenian pedo-climatic conditions, with full inflorescence maturity typically occurring only in (late) October. Slovenia has a continental climate, and October often brings substantial rainfall and fog, which complicate harvesting and diminish crop quality. Moreover, many foreign feminised varieties exhibited Δ^9 -THC concentrations near the upper legal limit of 0.3% in dry mass (European Commission, 2023), creating a persistent risk of exceedance under abiotic and biotic stressors (e.g., high temperatures, drought, and pest pressure) known to elevate THC concentrations (de Prato et al., 2022; Chandra et al., 2011). In response, a breeding programme for high-CBD, feminised industrial hemp was initiated in 2023 at the Biotechnical Faculty of the University of Ljubljana with two primary objectives: (i) to stabilise

Δ^9 -THC content in female inflorescences below 0.3 %, and (ii) to ensure timely maturation of female inflorescences, enabling punctual harvest and high product quality. These objectives reflect the need to develop cultivars adapted to Slovenia's specific pedo-climatic conditions.

Pruning is widely used in horticultural crops to manipulate apical dominance, redistribute assimilates, and enhance lateral branching (Cline, 1997). In hemp, pruning is thought to promote a bushier growth habit, potentially increasing the number of inflorescences and improving harvestable flower yield. Additionally, changes in canopy density may affect microclimate—light penetration, temperature, and humidity—thereby influencing cannabinoid biosynthesis (Dilena et al., 2023). However, research on pruning in hemp has produced mixed results. Some studies report increased branching and higher total flower yield (Caplan et al., 2017), whereas others indicate that pruning does not influence inflorescence yield per unit cultivation area, regardless of plant density (Danziger and Bernstein, 2022).

By altering plant morphology and source-sink relationships, pruning may affect cannabinoid accumulation both directly and indirectly. Increased branching can shift the proportion of secondary inflorescences, which may differ in cannabinoid concentration from apical flowers (Bernstein et al., 2019). For example, Massuela et al. (2022) found that pruning increased inflorescence biomass but did not raise total CBD yield because the CBD concentration of additional side branches was lower than that of apical inflorescences. Empirical evidence for industrial hemp remains limited, inconsistent, and highly dependent on genotype, environmental conditions, and production system. Notably, most available studies focus on drug-type cannabis grown indoors (Caplan et al., 2017; Backer et al., 2019), whereas field-grown industrial hemp—particularly feminised lines bred for CBD production—has received far less scientific attention.

Sowing date is one of the most influential agronomic decisions affecting floral biomass formation and cannabinoid accumulation in *C. sativa*, with recent studies consistently demonstrating that earlier establishment enhances both vegetative development and floral yield. Coolong et al. (2023) showed that planting date strongly modulates the balance between vegetative growth duration and the onset of reproductive development in both day-neutral and photoperiod-sensitive genotypes. Earlier sowing extended the vegetative phase, producing larger plants with greater floral biomass and higher total cannabinoid yield, whereas delayed planting compressed the growth pe-

riod and reduced both flower mass and cannabinoid concentration. Similar temporal effects have been observed in transplant-based systems: Linder et al. (2022) reported that earlier transplanting significantly increased floral biomass production, indicating that the timing of field establishment—whether by direct sowing or transplanting—plays a comparable physiological role in determining final inflorescence output. Likewise, García-Tejero et al. (2019) demonstrated that sowing date strongly influences both biomass production and cannabinoid yield in industrial hemp: early sowing increased total above-ground biomass by 20–35 % relative to later dates due to a longer vegetative period and greater canopy development. This extended growth window also enhanced secondary-metabolite formation, with early-sown plants producing 15–25 % higher CBD concentrations for the cultivar ‘Ermeš’ and substantially greater total cannabinoid yield per hectare. In contrast, late sowing shortened the growth cycle, accelerated flowering, and reduced both biomass and cannabinoid accumulation. Overall, these results highlight that optimising sowing date is essential for maximising hemp productivity and biochemical quality for biomedical applications.

The aim of the study was to evaluate Slovenian breeding lines of feminised industrial hemp under production-scale field conditions. Specifically, we investigated how combining agronomic interventions—pruning and sowing date—affects both productivity and phytochemical quality in four Slovenian breeding lines of feminised industrial hemp.

2 MATERIALS AND METHODS

2.1 CROSSING OF PARENTAL GENOTYPES TO OBTAIN BREEDING LINES

As seed production for breeding lines is not the primary focus of this manuscript, only limited information on the parental crosses is disclosed; detailed crossing methodologies are beyond the scope. To obtain breeding lines of feminised hemp, controlled crosses were conducted in 2023 using four feminised, CBD-rich *Cannabis sativa* L. genotypes (designated G8, G10, MER, and CH) as maternal parents, and a single industrial hemp genotype (female) from the variety ‘Tiborszallasi’ (designated T6) as the pollen donor. Male flowers on T6 were induced using silver thiosulfate as described in Flajšman et al. (2021). Seeds from each maternal plant genotype were harvested manually and stored for sowing in the subsequent year. Breeding line names correspond to their respec-

tive maternal genotypes with the suffix “c” (denoting “cross”), yielding the designations G8c, G10c, MERc, and CHc.

2.2 PLANT MATERIAL AND FIELD TRIALS

The experiment began on 6 May 2024 in the greenhouse of the Biotechnical Faculty, University of Ljubljana. Seeds from the four industrial hemp breeding lines were sown in 40-cell seedling trays. On the same day, vegetative cuttings were taken from lateral shoots of the mother-plant genotypes (G8, G10, MER, CH, T6), following the procedure described by Bitežnik et al. (2024). Cuttings of the parental genotypes were transplanted at the Ljubljana location and were used for biochemical comparison with the progeny breeding lines.

2.2.1 Field trials in Ljubljana and Krško

At both locations, fields were prepared in the same manner. The soil was ploughed and fertilised with 300 kg ha⁻¹ NPK (6:12:24), followed by secondary cultivation with a rotary harrow. An agro-textile mulch with pre-cut planting holes was laid over the prepared area, with an in-row spacing of 1.0 m and an inter-row spacing of 1.2 m, resulting in a planting density of 8,333 plants per hectare. This spacing allows each plant sufficient space to grow to its full potential without inter-plant interference. During the growing season, routine management included manual weeding around the planting holes and mechanical weed control between rows. No irrigation or additional fertilisation was applied during the growing season.

The experiments followed a completely randomised design with a 4 × 2 × 2 factorial arrangement and 10 plants per treatment (n = 160 breeding-line plants per location). The factors were: (i) breeding line (G8c, G10c, MERc, CHc), (ii) pruning (topping = cutting of apical meristem, and no topping-control), and (iii) sowing date/seedling establishment method (seedling production (young plants) in the greenhouse, followed by transplanting to the field and direct sowing of seeds into the field). At the Ljubljana site, parental genotypes were also cultivated. Additional details of the field experiments are provided in Table 1.

2.3 SAMPLING AND MEASUREMENTS

At harvest, plant height was measured. The apical 10 cm of the main inflorescence was then excised, and the remaining inflorescences were stripped from the stem. Drying was carried out in a drying chamber at a

Table 1: Soil physicochemical characteristics and the timeline of agronomic measurements conducted at each location

	Ljubljana	Krško
Exact location	Laboratory Field of the Biotechnical Faculty, Jamnikarjeva 101, Ljubljana	village Kalce-Naklo, located 10 km southern of town Krško, GERK number of the field: 5233527
Soil characteristics	clay loam, heavy soil pH: 6.8 Organic matter: 3.9% P_2O_5 : 29.5 mg/100 g (FE: D) K_2O : 15.9 mg/100 g (FE: B)	medium-heavy to heavy soil pH: 6.6 Organic matter: 3.1% P_2O_5 : 17.9 mg/100 g (FE: C) K_2O : 22.5 mg/100 g (FE: C)
Direct field sowing	20 May 2024	19 May 2024
Planting of seedlings (sown on 6 May 2024) to the field	25 May 2024	25 May 2024
Planting of parental genotypes cuttings (made on 6 May 2024) to the field	20 June 2024	/
Seedling thinning to 1 plant/ planting hole	16 July 2024	24 June 2024
Topping of breeding lines (10 plants per line)	16 July 2024	27 June 2024
Harvesting of plants	Breeding lines: from 3 September 2024 to 20 September 2024 Parental genotypes: 26 September 2024	Breeding lines: 30 September 2024 Parental genotypes: /

FE - fertility class (Mihelič et al, 2010)

constant temperature of 38 °C for one week. Following drying, inflorescences were weighed to determine flower yield (g per plant), and the main inflorescence was retained for biochemical analysis.

The dried main inflorescences were ground to a fine powder. For cannabinoid extraction, 0.200 g of ground material was weighed into centrifuge tubes, and 10 ml of 96 % refined ethanol (Stella Technology) was added. After 15 min of incubation at room temperature, a 2 ml aliquot of each extract was filtered through a 0.45 µm membrane filter and transferred to vials.

Samples were analysed on a FOCUS GC (Thermo Scientific) equipped with an autosampler and a flame-ionisation detector (FID). Separation was performed on a TG-35MS fused-silica capillary column (Thermo Scientific; 30 m × 0.25 mm i.d.; 0.5 µm film). One microlitre of extract was injected in split mode (split ratio 30:1) at an inlet temperature of 250 °C. Ultrapure helium was used as the carrier gas at a constant flow of 2.0 mL min⁻¹. The GC oven tem-

perature program started at 150 °C (1 min), followed by a linear gradient of 15 °C/min to 350 °C, temperature was then kept constant for 5 min. The FID temperature was set to 320 °C.

Analytes were identified using retention indices (RIs) and comparison with external standards, and quantified by external calibration based on peak areas and sample mass. The cannabinoids analysed included CBDV (cannabidivarin), CBC (cannabichromene), CBD (cannabidiol), THC-Δ8 (delta-8-tetrahydrocannabinol), THC-Δ9 (delta-9-tetrahydrocannabinol), and CBG (cannabigerol). In this manuscript, only CBD and THC-Δ9 (THC) are reported.

Environmental data were obtained from the official website of the Slovenian Environmental Agency (ARSO, 2026). Weather conditions for both locations are presented in Fig. 1.

At the Ljubljana site, temperatures in 2024 were higher than the 1991–2020 long-term mean in every

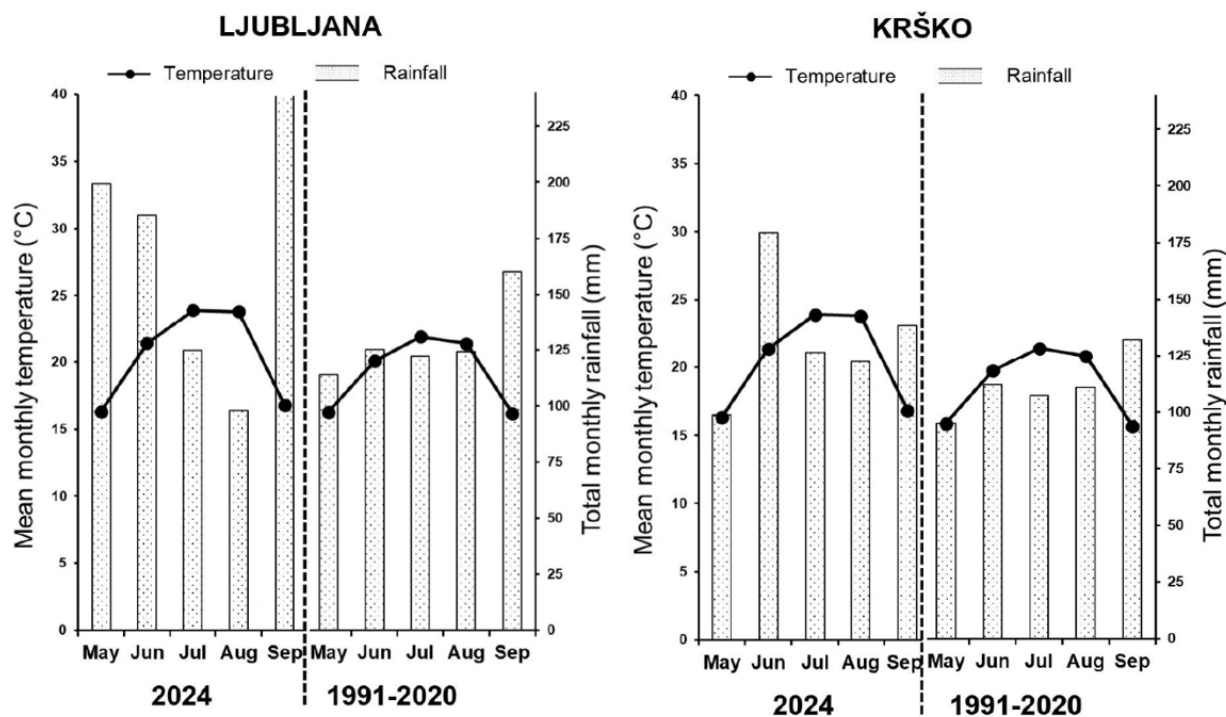


Figure 1: Mean monthly temperature and total monthly precipitation from May to September for the field experiments in year 2024 and the long-term average (1991-2020) for location Ljubljana – left (data obtained for weather station Ljubljana Bežigrad) and location Krško – right (data obtained for weather station Airport Cerklje ob Krki). The ratio between precipitation and temperature on the y-axes is set to 6 mm per 1 °C, reflecting typical climatic conditions in Slovenia. When the temperature curve exceeds the precipitation bars, it indicates the likelihood of mild drought conditions.

month. The largest positive anomalies occurred in July and August, when the mean temperature reached 24.4 °C in both months, compared with long-term means of 21.8 °C (July) and 21.3 °C (August). In June and September, the anomalies were smaller. With respect to precipitation, total rainfall in May and June 2024 was considerably higher than the long-term mean. In July, totals were nearly identical across the two periods at approximately 125 mm. In August, however, precipitation in 2024 was markedly lower than the reference period. The greatest difference was observed in September, when 247.5 mm was recorded in 2024, far exceeding the long-term mean (160 mm).

At the Krško site, monthly mean temperatures in 2024 exceeded the long-term mean throughout the field experiment, with the most pronounced deviations in July and August. Differences in May and September were smaller but still evident, indicating that summer 2024 was markedly warmer than average. Total monthly precipitation in Krško in 2024 also exceeded the long-term mean in every month. The highest total occurred in June, exceeding the long-term mean by approximately 60 %, whereas the

lowest total was recorded in May. Unlike the experiment in Ljubljana, the greater precipitation in Krško fell early in the growing season and therefore did not cause waterlogging during flowering or harvest.

2.4 STATISTICAL ANALYSES

At each location, the field trial was analysed as an independent experiment. Prior to analysis, each response variable was tested for homogeneity of variances using Levene's test. A three-way ANOVA was conducted to assess the main effects of breeding line, sowing date, and pruning, and their interactions, on the measured variables. When significant effects were detected, means were compared using Duncan's post hoc test at $\alpha = 0.05$, with significant differences indicated by different letters according to the compact letter display procedure. All analyses were performed in R version 4.3.1 (R Core Team, 2023) using the packages 'rcomdr' and 'agricolae'. Data manipulation and graphical presentation were carried out in Microsoft Excel programme.

3 RESULTS AND DISCUSSION

Weather and soil conditions strongly influenced the outcomes of the field experiments conducted during the 2024 growing season (May–September) at both locations, particularly in Ljubljana. The soils at this site contain up to 45 % sand and are classified as very heavy. The exceptionally high precipitation in May and June 2024 in Ljubljana (Fig. 1) caused prolonged surface water retention (waterlogging) and subsequent plant mortality, reducing the number of plants included in the statistical analysis to a maximum of seven per treatment, rather than the ten originally planned. At the Krško site, unusually high precipitation occurred only in June; however, the plant stand was not affected.

3.1 ANOVA ANALYSIS

Four variables were analysed using a three-way ANOVA. The factors considered were breeding line (BL), sowing date (SD), and pruning (P). The results are presented in Table 2. Plant height was the most affected variable: at Krško, all main factors and the BL $\times$ SD interaction were statistically significant; a similar pattern was observed in Ljubljana, except that SD alone was not significant. Flower yield showed a comparable response at both locations, with SD and the BL $\times$ SD interaction being statistically significant. BL and SD had moderate effects on CBD and THC contents, with no interaction detected. No three-way interaction (BL $\times$ SD $\times$ P) was observed for any variable at any location.

3.2 PLANT HEIGHT

Location had evident effect on plant height. Across all factors, plants were consistently taller in Krško than in Ljubljana (e.g., by breeding line: 231–167 cm vs 138–

98 cm; by sowing date: 235 vs 116–118 cm; by pruning: 228–210 vs 125–105 cm), indicating a strong location effect on vegetative growth (Fig. 2). Location driven absolute plant height, plausibly via excess moisture at Ljubljana. The systematic height depression in Ljubljana is consistent with reports that excess rainfall and waterlogging reduce hemp growth through root hypoxia and suppressed photosynthesis, particularly when saturation occurs in vegetative to early flowering stages (Kumar et al., 2025). This mechanism is also broadly supported by crop-physiology syntheses on waterlogging stress (Zhang et al., 2025) and hemp extension guidance highlighting the disease-conducive, yield-limiting effects of standing water (Schwartz and Smart, 2024).

Plant height differed markedly among breeding lines, sowing dates, and pruning treatments at both experimental locations. In Krško, the tallest plants were observed in MERc (231 cm), G10c (226 cm), and CHc (221 cm), which did not differ significantly from each other, while G8c produced significantly shorter plants (167 cm). In Ljubljana, MERc remained the tallest line (138.2 cm), followed by CHc (118.1 cm), whereas G10c (97.6 cm) and G8c (107.3 cm) formed a separate group of shorter plants (Fig. 2). Breeding lines differed in height at both sites, with rank changes (e.g., G10c: tall in Krško but shortest in Ljubljana), indicating crossover $G \times E$ (genotype $\times$ environment). Multi-environment trials show plant height in hemp is strongly affected by the environment and $G \times E$, even when some traits (e.g., phenology or cannabinoids) are more genetically stable (Campbell et al., 2019; Gill et al., 2025). Similar conclusions emerge from broader agronomy reviews emphasizing location-specific adaptation (Sebastian et al., 2023).

Sowing date also significantly influenced plant height. In Krško, direct sowing resulted in substantially taller plants (235 cm) compared to seedling transplanting (185 cm). In contrast, in Ljubljana, both sowing dates produced similar plant heights (116–118 cm), indicating

Table 2: Three-way ANOVA of four variables for locations Ljubljana and Krško

	Plant height		Flower yield		CBD content		THC content	
	LJ	KK	LJ	KK	LJ	KK	LJ	KK
Breeding line (BL)	***	**	ns	ns	***	***	ns	***
Sowing date (SD)	ns	***	*	*	ns	ns	ns	**
Pruning (P)	**	*	ns	ns	ns	ns	ns	ns
BL $\times$ SD	***	*	**	*	ns	ns	ns	ns
BL $\times$ P	ns	ns	ns	ns	ns	ns	ns	ns
SD $\times$ P	ns	ns	ns	ns	ns	ns	ns	ns
BL $\times$ SD $\times$ P	ns	ns	ns	ns	ns	ns	ns	ns

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns - not significant; LJ – location Ljubljana; KK – location Krško

no detectable sowing-date effect under the wetter conditions at this site (Fig. 2). In Krško, direct sowing produced taller plants than transplanting, aligning with some other field trials showing that earlier establishment (earlier transplant dates/sowing) yields taller, wider plants and higher biomass (Linder et al., 2022; Bajwa et al., 2025; Adamovics et al., 2017). The absence of a sowing date effect in Ljubljana suggests that environmental constraints (e.g., episodic surface water) likely overrode establishment advantages, a phenomenon anticipated when waterlogging diminishes growth potential.

Pruning had a consistent effect across locations. In Krško, non-topped plants were significantly taller (228 cm) than topped plants (210 cm). The same pattern was observed in Ljubljana, where non-topped plants reached 125 cm compared with 105 cm in topped plants (Fig. 2). The reduction in plant height with topping is in line with hypothesis, since topping reduces the high not only physically, but also via apical-dominance disruption (Cao et al., 2023). Our results that topping reduced height by ~18–20 cm at both sites mirrors other hemp studies where topping lowered plant height (Kocjan-Ačko et al., 2019; Roussis et al., 2022; Massuela et al., 2022). The mechanism fits classic apical dominance physiology—topping relaxes auxin-mediated suppression of axillary buds and alters auxin/strigolactone/sugar signaling, promoting lateral growth and a shorter habit (Mason et al., 2014; Beveridge et al., 2023).

A statistically significant interaction between breeding line (BL) and sowing date (SD) was observed for plant height at both locations. This interaction is reflected in the contrasting performance of specific BL × SD combinations: direct sowing produced the tallest plants for G10c (247 and 119 cm), CHc (246 and 119 cm), and MERc (243 and 135 cm) in Krško and Ljubljana, respectively, whereas seedling transplanting resulted in the lowest plant heights for CHc in Ljubljana (171 cm) and for G10c in Krško (57 cm) (Supplementary Fig. 1). These contrasting responses indicate that the effect of sowing date depends on breeding line, confirming the presence of a BL × SD interaction.

In conclusion, the results demonstrate that plant height was strongly influenced by breeding line and pruning at both sites, while the effect of sowing date was site-specific—pronounced in Krško but absent in Ljubljana. Moreover, the interaction between breeding line and sowing date highlights variability in the genetic background of the tested lines and emphasizes the importance of genotype-specific responses to establishment method. G8c stood out as the most promising breeding line, having the lowest height, since tall plants are not desirable for cannabinoid production. Topping also proved to be an effective tool for reducing plant height.

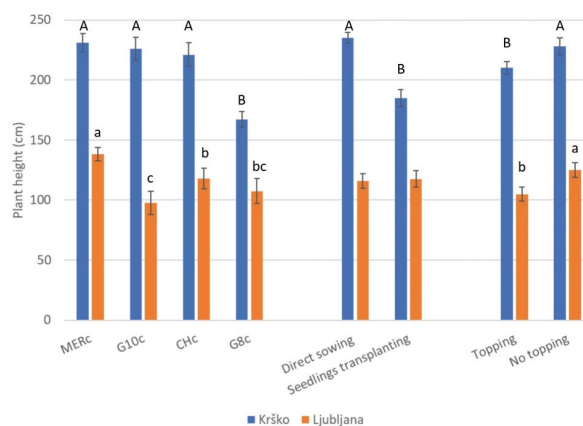


Figure 2: The plant height for locations Krško and Ljubljana, grouped by factor breeding line (MERc, G10c, CHc and G8c), sowing date (Direct sowing and Seedlings transplanting) and pruning (Topping and No topping). The upper case letters indicate statistically significant differences within each factor separately for location Krško and lower case letters indicate statistically significant differences within each factor separately for location Ljubljana. No letters mean no statistically significant differences between means within each factor. Error bars indicate standard errors of the mean values

3.3 FLOWER YIELD PER PLANT

Only sowing date of all three main factors (other two are breeding line and pruning) had statistically significant effect on the flower yield at both locations, which responded differently across locations. In Krško, direct sowing produced a higher mean yield (227 g plant⁻¹) than seedling transplanting (192 g plant⁻¹), a 15.8 % decrease with transplanting. In contrast, in Ljubljana seedling transplanting outperformed direct sowing, with 36 vs 25 g plant⁻¹, corresponding to a 43 % increase. These opposite responses indicate a location-specific effect of establishment method (Fig. 3). The Ljubljana advantage of transplanting (e.g., +43 % over direct sowing at the main-effect level) is consistent with stand-establishment literature showing that early-season stresses—cool soil, heavy rainfall, soil crusting, or pathogen pressure—can depress emergence and uniformity in direct sowings, thereby lowering yield; vigorous transplants buffer these constraints and stabilize stands (Cantliffe, 1989; Grassbaugh and Bennett, 1998; Finch-Savage and Bassel, 2016). It is evident that the high amount of rainfall in May and June after direct sowing in Ljubljana very negatively affected establishment and subsequent plant growth, consistent with the well-documented effects of waterlogging on seedling emergence and early growth through root hypoxia and reduced soil aeration (Kaur et al., 2020; Langan et al., 2022). On the other hand, transplanted seed-

lings were more successful in growth, probably due to their already established root systems and greater early vigor, which enabled them to better tolerate excessive soil moisture and reduced aeration (Kaur et al., 2020). Such transplant-related advantages—particularly improved early root development, enhanced shoot vigor, and higher early-season performance under limiting environmental conditions—are consistent with findings across horticultural crops (Leskovar and Othman, 2021; Gavrić and Omerbegović, 2021), whereas direct seeding can perform as well or better when emergence is rapid and transplant shock is minimal (Maboko and Du Plooy, 2014).

By contrast to Ljubljana, in Krško direct sowing was favoured—an outcome expected where warmer and well-drained soils permit rapid emergence and undisturbed taproot development that supports greater reproductive biomass (Grassbaugh and Bennett, 1998). In some crops and environments, direct-sown plants surpass transplants because they avoid transplant shock and potential root system constraints (e.g., circling, limited root exploration) that can occur with containerized transplants (Leskovar and Othman, 2021). Indeed, June received high amount of precipitation in Krško (180 mm) which probably caused water stress for the transplanted seedlings and negatively affect their growth. On the other hand, plants emerging directly from seed were better able to establish under these conditions, likely because their root systems developed in situ without disturbance, enabling more effective acclimation to transient waterlogging and improved maintenance of root–soil contact necessary for resource acquisition. Our results also align with findings from medicinal crops where sowing date and establishment conditions critically determine flower yield. In *Calendula officinalis* L. for example, sowing timing and density significantly influence flower yield and harvest index, with earlier or optimally timed sowings generally maximizing floral biomass (Seghatoleslami and Mousavi, 2009; Mirzaei et al., 2016; Hashem, 2016).

Among the interactions between the main factors, only the interaction between breeding line (BL) and sowing date (SD) exhibited a statistically significant effect, and that was at both locations. In Krško, the highest yields were observed for combination G8c–Seedlings transplanting (255 g plant⁻¹), CHc–Direct sowing (247 g plant⁻¹), and MERc–Direct sowing (245 g plant⁻¹), all statistically indistinguishable from G8c–Direct sowing (221 g plant⁻¹), G10c–Direct sowing (203 g plant⁻¹) and G10c–seedlings transplanting (203 g plant⁻¹). CHc–seedlings transplanting yielded the lowest flower yield (123 g plant⁻¹). The highest combination in Krško (G8c–seedlings transplanting) more than doubled the yield of

the lowest (CHc–seedlings transplanting; +108 %). In Ljubljana, G8c–seedlings transplanting produced the highest flower yield (47 g plant⁻¹), followed by MERc–seedlings transplanting (38 g plant⁻¹), CHc–seedlings transplanting (38 g plant⁻¹), and MERc–Direct sowing (34 g plant⁻¹). The lowest yield was obtained with G8c–Direct sowing (7 g plant⁻¹), what is about 7.1-fold less than the best combination (Fig. 3). Breeding line × Sowing date interaction indicates genotypic plasticity to establishment conditions. Transplants often confer earliness and initial canopy advantages, yet they can underperform if transplant stress coincides with critical phenophases or if root exploration is limited; conversely, direct-sown plants benefit from uninterrupted root axis development, which can support higher per-plant biomass when emergence is uniform and timely (Leskovar and Othman, 2021). Seed/seedling-vigor reviews further show that rapid, uniform emergence is a primary determinant of downstream yield stability, and that field stresses around sowing magnify differences between establishment methods (Finch-Savage and Bassel, 2016; Cantliffe, 1989).

In conclusion, our experiments showed a location-specific response to establishment method: direct sowing outperformed transplanting in Krško, whereas seedling transplanting was superior in Ljubljana. Similar context-dependent advantages of establishment methods are widely reported in horticultural crops, where transplanting can increase early vigor and marketable yield when early growth is limiting, but direct seeding can prevail when soils are warm, emergence is rapid, and transplant shock or root restriction would otherwise reduce performance (Leskovar and Othman, 2021). However, weather conditions had severe impacts on plant growth at both locations, and the contrasting responses appear to be largely driven by differences in soil moisture. Across all treatment combinations, absolute flower yields were higher in Krško (average 211 g plant⁻¹) than in Ljubljana (29 g plant⁻¹) implying severe negative growing conditions in Ljubljana. Excess early-season rainfall in combination with very heavy soil in Ljubljana created waterlogged conditions that restricted aeration and hindered the establishment of directly sown plants, consistent with broader evidence that soil saturation limits root respiration, nutrient uptake, and early seedling development. By contrast, the warmer and better-drained soils in Krško favoured in-situ root development of directly sown plants, enabling rapid emergence and early vegetative growth despite episodic rainfall. Thus, the relative success of each establishment method appears to depend not only on crop physiology but also on the interaction between local edaphic conditions and short-term weather patterns.

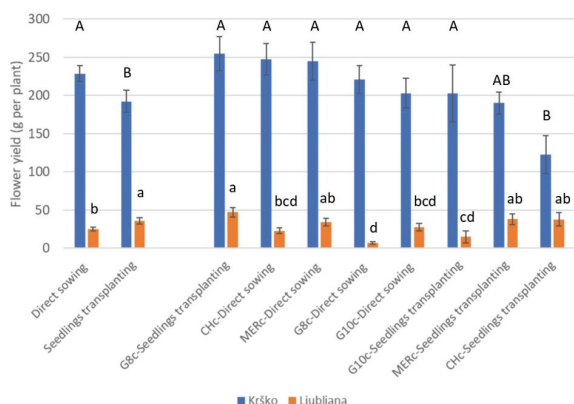


Figure 3: The flower yield for locations Krško and Ljubljana, grouped by factor sowing date (direct sowing and seedlings transplanting) and interaction breeding line x sowing date. The upper case letters indicate statistically significant differences within factor/interaction separately for location Krško and lower case letters indicate statistically significant differences within factor/interaction separately for location Ljubljana. Error bars indicate standard errors of the mean values

3.4 CBD CONTENT

Across locations, CBD content varied among breeding lines, whereas sowing date and pruning did not affect CBD content within either location. In Krško, CBD content differed among lines, ranging from 3.00 % to 5.02 %. The line MERc achieved significantly the highest CBD content (5.02 %), exceeding CHc (4.24 %) and G8c (3.99 %), which in turn were higher than G10c (3.00 %). In Ljubljana, MERc again produced the highest CBD (6.66 %). The remaining lines—G8c (5.21 %), G10c (4.69 %), and CHc (4.30 %)—did not differ from each other (Fig. 4). Taken together, MERc consistently outperformed the other lines at both sites, while G10c had the lowest CBD in Krško and intermediate CBD in Ljubljana. The pattern of the same genotype is having the highest CBD content is consistent with multi-environment trials indicating that cannabinoid production is largely under genetic control, even though many yield-related traits are more environmentally plastic (Campbell et al., 2019; Carlson et al., 2021; Sebastian et al., 2023). The stable rank order of lines across two locations fits reports that chemotype is predominantly genotype-driven, whereas biomass and phenology respond more strongly to environment (Toth et al., 2021; Sandhu et al., 2022; Naim-Feil et al., 2023). The absolute levels observed (≈ 3.0 – 6.7 % CBD) lie within the lower to mid-range for field-grown hemp. Surveys and germplasm evaluations report flower CBD up to ~ 8 % in diverse, outdoor-grown material (Alsaleh and Yilmaz, 2025) or up to 2 % under plastic macro-tunnels (García-Tejero et al., 2019), whereas some

controlled-environment or favourable field studies show ~ 6.5 – 12 % strongly depending on genotype and management (Coolong et al., 2023; James et al., 2023).

CBD content did not differ between sowing dates within either site. In Krško, direct sowing yielded 4.20 % and seedlings transplanting 3.77 %. In Ljubljana, CBD was 5.29 % for direct sowing and 5.27 % for seedlings transplanting (Fig. 4). No effect of sowing date on CBD content at both sites agrees with field experiments of Coolong et al. (2023), where cannabinoid concentration was affected by cultivar and year, but not by planting date for both photoperiod-sensitive and day-neutral cultivars. Other multi-site experiment likewise shows that planting date effects on CBD content are inconsistent and generally weaker than genotype effects, although earlier planting can increase floral biomass and CBD yield in some cultivars (Chiluwal et al., 2023). Comparable conclusions were drawn for CBG-dominant germplasm: location and planting date influenced profiles but not in a consistent, genotype-independent way (Chiluwal et al., 2023).

Pruning treatment had no detectable effect on CBD content within sites. In Krško, topping and no topping resulted in 3.98 % and 4.14 %, respectively. In Ljubljana, topping yielded 5.51 % and no topping 5.09 %. Our finding mirrors some other research conducted in controlled-environment and field/greenhouse. Apical cut can increase inflorescence biomass but often does not raise CBD concentration and frequently fails to increase total CBD yield vs unpruned controls (Massuela et al., 2022). In the study by Benevenuto et al. (2021), field trials with two high CBD cultivars similarly reported that pinching (topping) did not improve flower yield, cannabinoid concentration, or CBD production. Greenhouse work with a CBD-type cultivar found no cannabinoid or biomass benefit of pruning, reinforcing that pruning should be considered for canopy architecture and logistics rather than for quality per se (Dilena et al., 2023).

Means of CBD content were generally higher in Ljubljana than in Krško for most breeding lines (e.g., MERc: 6.66 % vs. 5.02 %; G8c: 5.21 % vs. 3.99 %; G10c: 4.69 % vs. 3.00 %), whereas CHc exhibited similar means across sites (4.30 % vs. 4.24 %). Although cross-site statistics were not performed here, the higher means in Ljubljana suggest environmental influence to the cannabinoid concentrations (e.g., photoperiod, temperature regime, light environment, etc.). Reviews and field observations emphasize that latitude/photoperiod and climate can shift flowering dynamics and absolute cannabinoid accumulation, even when the relative chemotype is genetically fixed (Sebastian et al., 2023; Rheay et al., 2025). Moreover, light quantity/quality and UV exposure are known to modulate secondary metabolites in hemp, which helps contextualize site-level differences in outdoor trials (De-

saulniers Brousseau et al., 2021). At the same time, multi-environment analyses find that CBD and THC concentrations tend to be more genetically stable across sites than many agronomic traits, which aligns with our stable ranking but site-shifted means (Campbell et al., 2019).

Plants gain more environmental stress in Ljubljana mainly due to excessive rain in Maj/June and consequently waterlogging which suppresses root oxygen status. Ethylene-mediated hypoxia acclimation tempers ROS damage, but mainly at the cost of growth (Liu et al., 2022), what was confirmed also in our experiment with plants of all breeding lines at Ljubljana being shorter and having lower flower yields. However, different CBD concentration cross locations in our study means that likely moisture stress caused higher CBD concentration in Ljubljana. This observation is contrary to irrigation study in semi-arid field hemp, which indicate that varying water availability within agronomic ranges changes biomass and floral yield, but not than cannabinoid concentrations (Roseberg et al., 2025), suggesting that moisture excess primarily affects quantity rather than potency except under severe or prolonged stress (Vijaya Kumar et al., 2025).

To conclude, genotype was the dominant driver of CBD concentration across both sites: the line MERc consistently produced the highest CBD, while G10c was lowest or intermediate depending on site. Sowing date and pruning had no detectable effect on CBD at either loca-

tion, indicating these practices mainly influence canopy or yield logistics rather than potency. Although absolute CBD levels were generally higher in Ljubljana than in Krško, the stable rank order among lines underscores strong genetic control of chemotype, with environment shifting magnitudes but not relative performance.

3.5 THC CONTENT

All observed THC means (0.12–0.21 %) were well below the EU's current on-field threshold of 0.3 %, which has applied since 1 January 2023 under the reformed Common Agricultural Policy; this threshold governs eligibility of hemp varieties for direct payments and effectively frames agronomic risk for exceeding this threshold in the EU (European Commission, 2023).

A significant breeding line effect was detected in Krško, where G10c exhibited significantly the lowest THC content (0.12 %), while there was no statically significant difference between lines MERc (0.17 %), CHc (0.15 %), and G8c (0.15 %). In Ljubljana, THC did not differ among breeding lines (0.16–0.21 %), indicating no detectable line effect at that site (Fig. 5). The observation, that breeding line had effect at one and no effect at other location is in line with evidence that THC potential is strongly genotype-bounded, yet genotypes differ in environmental sensitivity, producing detectable $G \times E$ (Campbell et al., 2019). In high-cannabinoid hemp, CBD and THC often co-accumulate through floral maturation, and genotype explains a large share of the variance, supporting the interpretation that inherently low-THC lines (e.g., G10c in Krško) help maintain compliance across sites (Stack et al., 2021).

Sowing date influenced THC content in Krško, with direct sowing yielding a higher THC content (0.16 %) than seedling transplanting (0.13 %). In Ljubljana, THC content was identical for the two sowing dates (0.19 % for both), indicating no sowing-date effect there. Evidence from other studies indicates that planting date exerts inconsistent effects on cannabinoid percentages, whereas genotype is the primary determinant, and yield responds more consistently to planting windows. Moreover, Coolong et al. (2023) reported that planting date did not affect cannabinoid concentrations in either day-neutral or photoperiod-sensitive cultivars. Similarly, Sandhu et al. (2022) found that varietal genetics explained more variance than planting date in cannabinoid profiles and emphasised the importance of harvest timing, as total THC increased over time and sometimes exceeded the 0.3 % threshold late in development.

Pruning did not affect THC content at either location. In Krško, THC was 0.15 % irrespective of pruning (topping vs. no topping). In Ljubljana, THC was numeri-

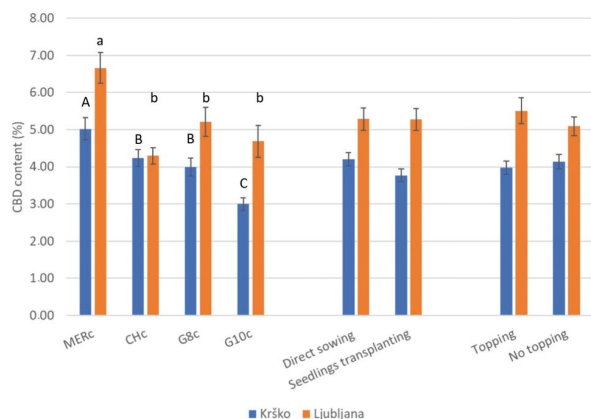


Figure 4: CBD content for locations Krško and Ljubljana, grouped by factor breeding line (MERc, CHc, G8c and G10c), sowing date (direct sowing and seedlings transplanting) and pruning (topping and no topping). The upper case letters indicate statistically significant differences within each factor separately for location Krško and lower case letters indicate statistically significant differences within each factor separately for location Ljubljana. No letters mean no statistically significant differences between means within each factor. Error bars indicate standard errors of the mean values

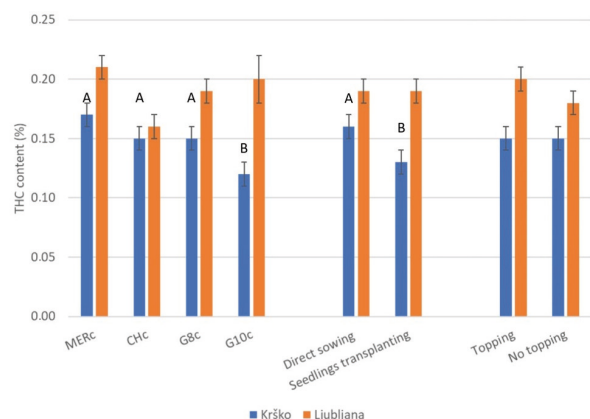


Figure 5: THC content for locations Krško and Ljubljana, grouped by factor breeding line (MERc, CHc, G8c and G10c), sowing date (direct sowing and seedlings transplanting) and pruning (topping and no topping). The upper case letters indicate statistically significant differences within each factor separately for location Krško. No letters mean no statistically significant differences between means within each factor. Error bars indicate standard errors of the mean values

cally higher with topping (0.20 %) than with no topping (0.18 %), but the difference was not statistically significant (Fig. 5). Null effect of topping on THC aligns with other research showing topping mainly alters architecture/biomass (and can reduce fiber yield when grown for fiber) without a consistent THC response (Bajić et al., 2022). Greenhouse pot experiments have reported higher CBD after topping (stress-related), but no uniform THC increase (Roussis et al., 2022).

Across all factor levels, THC values were consistently higher in Ljubljana than in Krško (Ljubljana: 0.16–0.21 %; Krško: 0.12–0.17 %) (Fig. 5) what is consistent with environmental modulation within genetic bounds. Heat stress can upregulate cannabinoid-pathway gene expression and increase decarboxylation products (Hahm et al., 2025), while water status and photoperiod-related physiology affect carbon allocation and sec-

ondary metabolism (Gill et al., 2022). Together, modest differences in temperature, moisture, and light between sites can move THC % within a cultivar's intrinsic range and obviously environment in Ljubljana enable plants to synthesized higher amount of THC.

To conclude, THC concentrations (0.12–0.21 %) at both locations and treatments remained below the EU on-field threshold of 0.3 %, confirming regulatory compliance. A genotype × environment signal was evident: in Krško the breeding line G10c showed the lowest THC, whereas no line differences appeared in Ljubljana, indicating environment-dependent expression of genotype. Sowing date only mattered in Krško (direct sowing > transplanting), while pruning (topping) had no effect at either site. Overall, Ljubljana produced slightly higher THC than Krško across factors, consistent with modest environmental modulation within genetic bounds.

3.6 COMPARISON OF CBD AND THC CONTENT OF PARENTAL GENOTYPES AND BREEDING LINES

Comparison of parental genotypes with their derived breeding lines revealed that most breeding lines exhibited reductions in CBD concentration. The parental genotype G10 contained 12.19 % CBD, whereas its descendant G10c produced 4.69 % CBD in Ljubljana (–62 %) and 3.00 % in Krško (–75 %). For the parental genotype G8 (11.59 % CBD), the line G8c declined to 5.21 % in Ljubljana (–55 %) and 3.99 % in Krško (–66 %). The parental genotype CH (11.14 % CBD) decreased to 4.30 % in Ljubljana (–61 %) and 4.24 % in Krško (–62 %) in the line CHc. In contrast, the parental genotype MER, which had the lowest CBD among the maternal parents (7.24 %), showed smaller reductions in the derived line MERc—to 6.66 % in Ljubljana (–8 %) and 5.02 % in Krško (–31 %). The paternal genotype T6 contained 4.37 % CBD (Table 3).

Regarding THC concentration, G10 exhibited the

Table 3: Comparison of average CBD content (plants with minimum and plant with maximum value are in brackets) of parental genotypes and breeding lines

Name	Average of parental genotypes (min, max) from Ljubljana (%)	Name	Average of breeding line (min, max) from Ljubljana (%)	Average of breeding line (min, max) from Krško (%)
G8 ¹	11.59 (7.34, 15.92)	G8c	5.21 (2.77, 7.69)	3.99 (1.50, 6.80)
G10 ¹	12.19 (9.22, 15.02)	G10c	4.69 (2.11, 8.16)	3.00 (0.85, 5.20)
CH ¹	11.14 (8.12, 13.24)	CHc	4.30 (2.08, 6.21)	4.24 (2.36, 7.36)
MER ¹	7.24 (4.10, 10.38)	MERc	6.66 (3.22, 11.18)	5.02 (2.17, 7.99)
T6 ²	4.37 (3.70, 7.09)	/	/	/

1 maternal genotypes; 2 paternal genotype

Table 4: Comparison of average THC content (plant with minimum and plant with maximum value are in brackets) of parental genotypes and breeding lines

Name	Average of parental genotypes (min, max) from Ljubljana (%)	Name	Average of breeding line (min, max) from Ljubljana (%)	Average of breeding line (min, max) from Krško (%)
G8 ¹	0.38 (0.24, 0.54)	G8c	0.19 (0.10, 0.28)	0.15 (0.06, 0.24)
G10 ¹	0.43 (0.34, 0.52)	G10c	0.20 (0.09, 0.39)	0.12 (0.06, 0.20)
CH ¹	0.39 (0.30, 0.47)	CHc	0.16 (0.08, 0.24)	0.15 (0.04, 0.24)
MER ¹	0.25 (0.15, 0.35)	MERc	0.21 (0.10, 0.36)	0.17 (0.09, 0.32)
T6 ²	0.14 (0.13, 0.24)	/	/	/

1 maternal genotypes; 2 paternal genotype

highest THC concentration (0.43 %) among the parental genotypes. Its derived line G10c showed substantial reductions, reaching 0.20 % THC in Ljubljana (–53 %) and 0.12 % in Krško (–72 %). The parental genotypes CH (0.39 %) and G8 (0.38 %) displayed similar THC levels; their corresponding lines contained 0.16 % (Ljubljana) and 0.15 % (Krško) for CHc, and 0.19 % (Ljubljana) and 0.15 % (Krško) for G8c, representing ~50–62 % decreases relative to the respective parents. The parental genotype MER had the lowest THC among the maternal parents (0.25 %); its descendant line MERc contained 0.21 % THC in Ljubljana (–16 %) and 0.17 % in Krško (–32%). The paternal genotype T6 contained 0.14 % THC (Table 4).

This study is describing new effort of hemp breeding in Slovenia. Industrial hemp breeding has a relatively short history in Slovenia. The first Slovenian monoecious industrial hemp variety, Fiona, was registered in 2021, followed by two additional varieties, Fortuna and Fia, registered in 2025—all bred at the Biotechnical Faculty of University of Ljubljana. In addition to these new releases, two Slovenian conservation hemp varieties are listed in the EU Plant Variety Database at the moment (Plant variety database ..., 2026), reflecting national efforts to preserve local genetic resources alongside ongoing improvement programmes.

In presented study, four breeding lines were evaluated in replicated field trials. Breeding lines were obtained by crossing high-CBD genotypes a single industrial-hemp genotype to generate feminized breeding lines. Because the parental genotypes were propagated clonally, whereas the lines were seed-propagated, direct comparisons of morphological traits of parental genotypes and its breeding lines were not undertaken. However, biochemical profiles were comparable, and the breeding lines exhibited reductions in both CBD and THC contents relative to their parents. The decline in THC is desirable for regulatory compliance and therefore advantageous for selection, whereas the concomitant reduction in CBD in-

dicates a strong parental influence on cannabinoid composition in the progeny. Notably, individual plants within each line displayed high CBD levels (Table 3), indicating that intra-line selection has the potential to recover superior chemotypes while maintaining low THC. A similar inter-type cross between industrial hemp and high-CBD hemp was recently reported by Zheljazkov et al. (2025), who hybridised a high-CBD U.S. cultivar with the dioecious European industrial hemp cultivar 'Marina'. Because the male parent was the industrial hemp cultivar, the cross did not produce feminised seed. Their progeny also showed substantial segregation for cannabinoid content, with CBD in dried material ranging from 0.207 % to 14.74 % and THC from 0.085 % to 0.447 %.

To conclude, four new Slovenian feminized breeding lines derived from high-CBD × industrial-hemp crosses exhibited reduced CBD and THC relative to their parental clones. The decrease in THC is advantageous for regulatory compliance, whereas the decrease in CBD underscores the need for continued breeding improvement. Nevertheless, the presence of high-CBD individuals within each line indicates that targeted intra-line selection can recover superior high-CBD/low-THC phenotypes, consistent with the broad segregation reported in comparable inter-type crosses. Further research will focus on selecting the best genotypes within each breeding line or on using these lines for new crosses.

4 ACKNOWLEDGEMENTS

The authors thank Mr Anže Vrhovec for his assistance with the field experiments at the Ljubljana site and his help with sample preparation. At the Ljubljana site, the field experiment was conducted at the Laboratory Field, supported by the infrastructural centre IC RRC-AG (IO-0022-0481-001). This research was supported by Grants from the Slovenian Research Agency, research programme P4-0077.

5 DATA AVAILABILITY

The data underlying this study are openly available in the Repository of the University of Ljubljana at <https://repozitorij.uni-lj.si/IzpisGradiva.php?id=179434> (Flajšman and Eržen, 2025)

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