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Enhancing yield of cannabis inflorescences and cannabinoids through plant stem infusion of sucrose: A novel cannabis cultivation approach

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ABSTRACT

The rising demand for cannabis, both medicinal and recreational, requires innovative cultivation methods to maximize yield and cannabinoid production. This pilot study investigated the potential of plant stem infusion of sucrose (PSIS) as a novel approach using precise control of infusion pressure and sucrose concentration to improve cannabis growth, morphology, physiology, and cannabinoid yield. Cannabis plants infused with sucrose under low pressure (0.5 bar) and high sucrose concentrations (15–30 %) exhibited significant improvements in flower dry mass (up to 31 %) and cannabinoid yield (up to 34 %) compared to control plants. Morphological analysis revealed that plants treated at 0.5 bar exhibited increased plant height and significantly greater flower and stem dry mass, while leaf biomass was reduced across all treated groups. Physiologically, respiration increased significantly under 1 bar pressure, likely due to metabolic responses to exogenous sucrose. However, net carbon assimilation, stomatal conductance, chlorophyll content, and other photosynthetic parameters showed no significant differences compared with the control. Our findings highlight that PSIS at an optimal pressure of 0.5 bar and sucrose concentration of 15–30 %, is an effective method for increasing cannabis flower mass and cannabinoid yield. While high pressures negatively impacted plant morphology and physiology, low-pressure infusion proved beneficial. This study establishes PSIS as a promising innovation in cannabis cultivation, warranting further research to optimize pressure and sucrose concentrations for even greater improvements in yield and quality.

1. Introduction

Cannabis sativa L., commonly known as hemp or cannabis, has emerged as an important crop with significant economic and medicinal value. The demand for high-quality cannabis has surged in both the medicinal and recreational markets and the rapid growth and intense competition are driving producers to find new ways to enhance yield, quality, and production efficiency (Small, 2017). Maximizing yield and enhancing the quality of cannabis plants, particularly by increasing the content of secondary metabolites such as cannabinoids, is paramount for meeting this growing demand.

Cannabis is used in many different applications, but its secondary metabolites are becoming increasingly important in the medical community. The most important are the phytocannabinoids, with cannabidiol (CBD) and trans-Δ9-tetrahydrocannabinol (Δ9-THC) being the most abundant and best characterized. Terpenoids are another important group of plant metabolites from cannabis and include limonene, safranal, geraniol, α-curcumene, α-selinene and farnesol (Mostafaei Dehnavi et al., 2022). Based on the composition of phytocannabinoids, cannabis is categorized into five different chemotypes: THC chemotype I (high THC, low CBD); intermediate chemotype II (ratio of THC and CBD close to 1); CBD chemotype III (high CBD, low THC); chemotype IV (CBG is the predominant cannabinoid); and chemotype V (no detectable cannabinoids) (Salami et al., 2020).

Achieving high yields and superior quality in cannabis cultivation involves overcoming several challenges. Traditional methods of nutrient delivery, such as drenching, fertigation, and foliar feeding, even when paired with plant-growth-promoting rhizobacteria, often fall short of

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Table 1
Plant utilization for two experimental factors (sucrose concentration vs pressure).

Sucrose concentration vs. applied pressure	0 %	7.5 %	15 %	30 %	TOTAL
0.5 bar	3	6	6	6	21
1 bar	3	6	6	6	21
2 bar	3	6	6	6	21
Negative control	-	-	-	-	9
TOTAL	9	18	18	18	**72**

Fig. 1. Vertical PVC tubes for medium connected to a compressor.

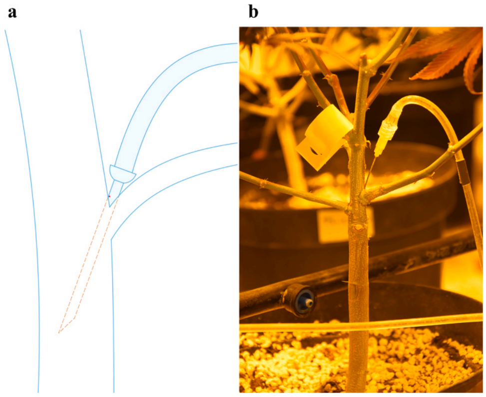


Fig. 2. Injection of the needle in cannabis stems. A schematic diagram of the injection process (**a**) and a photograph taken during the experiment (**b**).

maximizing plant production potential due to limitations in nutrient absorption and distribution (Backer et al., 2019). Consequently, growers seek innovative techniques to improve plant health, increase biomass, and enhance cannabinoid profiles.

One of the lesser-known methods for delivering nutrients, pesticides, vitamins, hormones, etc., into plants is by plant stem infusion (PSI). It has been explored and found to improve overall plant health in various species. For example, boron and calcium infusions in soybeans have significantly stimulated yield by increasing pod and seed development, indicating improved plant productivity (Schon and Blevins, 1987). In salt-stressed chickpeas, PSI has been shown to enhance drought tolerance and overall vigor (Khan et al., 2016). Tree injections have been effectively used to manage Dutch elm disease (Postma and Goossen-van de Geijn, 2016), oak wilt (Koch et al., 2010), emerald ash borer (Flower et al., 2015), apple scab (Aćimović et al., 2016), and more, providing protection and improving the health of trees. Moreover, PSI has been employed for pesticide delivery in *Lophostemon confertus* (Brisbane box) trees to manage damage from the caterpillar, *Uraba lugens*, demonstrating the potential for pest control (Rolando et al., 2011). These studies suggest that PSI can be effective in bolstering plant health by improving critical nutrient uptake and resistance to stress.

Plant stem infusion can also be used to deliver nutrients, but this poses some particular challenges. Although it is more effective than foliar feeding (Zhou and Smith, 1996), the efficiency of infusion, measured by the total volume of the injected infusion solution, strongly depends on applied pressure, solute content (concentration and osmolarity), pH, number of injection sites, and salt stress (Abdin et al., 1998; Boyle et al., 1991; Khan et al., 2016; Zhou et al., 1997, 1999; Zhou and Smith, 1996). The highest rate of infusion typically occurs within the first 24 hours, after which it declines sharply (Boyle et al., 1991; Ma et al., 1995).

Sucrose is the most commonly used nutrient in studies of PSI. Plant stem infusion of sucrose (PSIS) has been explored as a method to enhance plant growth and increase yields in various crops, including maize (Boyle et al., 1991; Zhou et al., 1999; Zhou and Smith, 1996), barley and wheat (Ma et al., 1995), soybean (Abdin et al., 1998), sweet potato (Kadowaki et al., 2001; Tsubone et al., 2000), chickpea (Khan et al., 2016), and bonsai trees (Zhang et al., 2023). Sucrose infusion aids plant growth by providing an additional source of energy and carbon as well as acting as a signal molecule, thereby influencing various physiological processes (Yoon et al., 2021). Carbohydrates, including sucrose, can induce osmotic or carbonyl stress, which can affect secondary metabolism and enhance the production of specialized metabolites (Skała et al., 2022). Sucrose also influences primary metabolic pathways, cellular growth, and differentiation, which are key processes for increasing biomass production and overall yield (Aluko et al., 2021). Sucrose also regulates the activity of many genes associated with carbohydrate metabolism, such as sucrose non-fermenting-1-related protein kinase-1 (SnRK1), which is responsible for sucrose, starch production, and enzymatic activity. Overexpression of SnRK1 in transgenic tobacco plants led to increased levels of sucrose, glucose, and fructose, directly contributing to plant growth and biomass production (Coleman et al., 2010; Wang et al., 2017). Generally, PSIS increases yield and downregulates photosynthetic activity (Khan et al., 2016; Tsubone et al., 2000; Zhou et al., 1999; Zhou and Smith, 1996).

The technology of stem infusion has developed over time. Initially, Grabau (Grabau et al., 1986) and Schon (Schon and Blevins, 1987) used standard medical intravenous kits with 21-gauge hypodermic needles to deliver treatment solutions to plants. Boyle (Boyle et al., 1991) introduced a modified system using a syringe with a 20-gauge needle injected through a rubber septum into the stem. Subsequent studies (Abdin et al., 1998; Khan et al., 2016; Zhou et al., 1999; Zhou and Smith, 1996) adopted Boyle's method, which remained rudimentary and lacked precise control over experimental parameters such as pressure. Our approach, used in this study, is similar to Boyle's system, but refines it by using an industrial air compressor to fine-tune pressure and sucrose concentration for each plant, allowing precise adjustment of parameters and better control over the infusion process.

The stem infusion method has been known for decades, but to the best of our knowledge, no one has used it with cannabis, therefore, this work is the first to investigate the effect of PSIS on the physiology and morphology of cannabis. We evaluated the potential of PSIS for

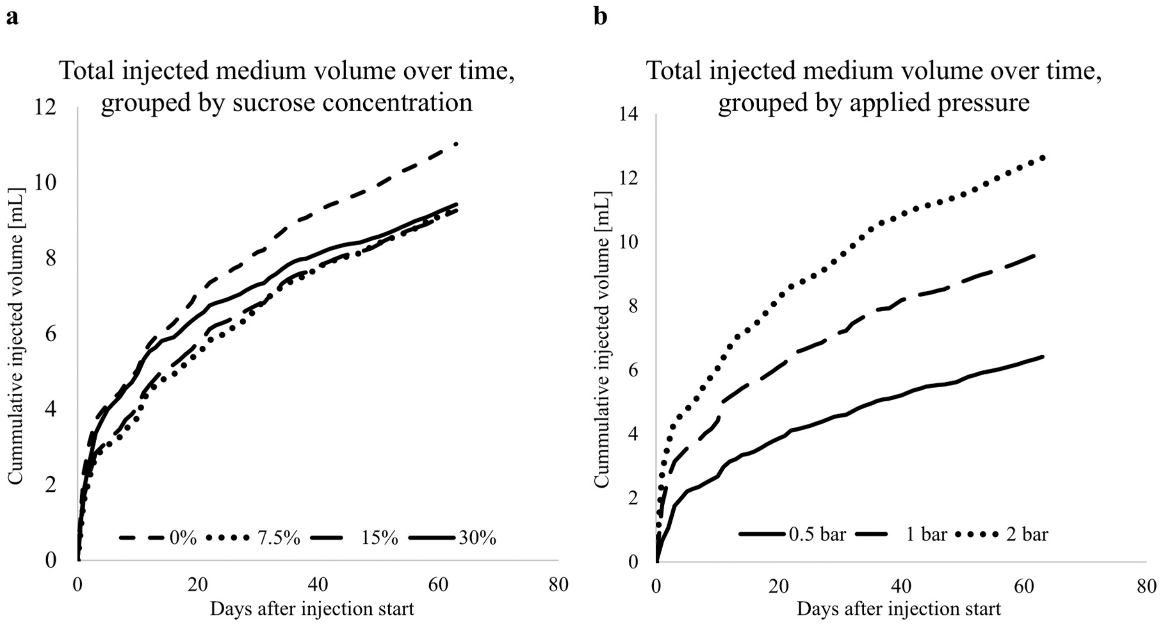


Fig. 3. Total average injected medium over time. Averages were calculated for the different sucrose concentrations (**a**) and the applied pressures (**b**).

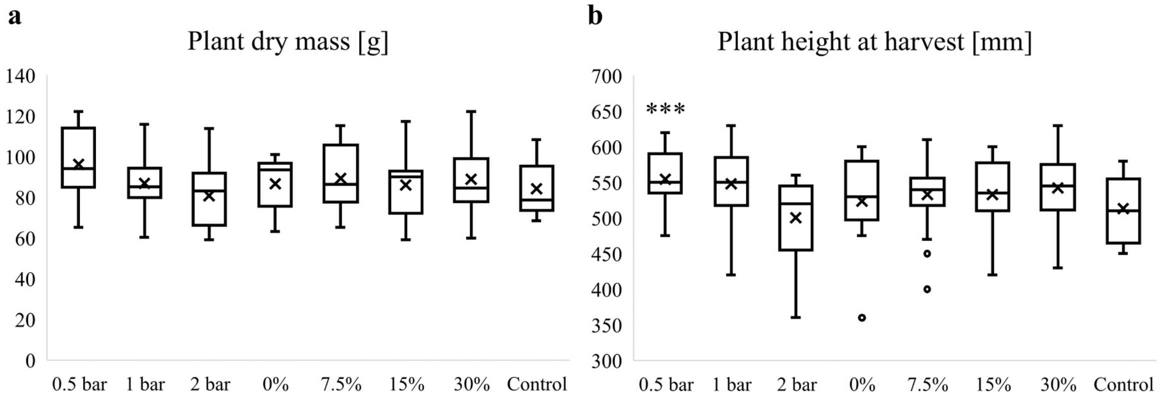


Fig. 4. Box plot comparisons of dry plant mass (**a**) and height at harvest (**b**). Plants were grouped based on either applied pressure or sucrose concentration in the infusion solution. The negative control group contained nine plants, while each applied pressure group contained 21 plants, and each sucrose concentration group contained 18 plants, except the group with 0 % sucrose, which only contained nine plants. Each group was compared to the negative control. A two-way ANOVA with Tukey's post-hoc test was used to calculate statistical significance: $*p < 0.1$, $* *p < 0.05$, $* **p < 0.01$.

increasing cannabis yields in terms of its effect on total plant dry mass, flower dry mass, and cannabinoid yield. We aim to identify optimal levels of applied pressure and sucrose infusion solution concentrations to maximize these parameters.

2. Materials and methods

2.1. Experimental design

Two primary variables were assessed: the concentration of the sucrose solution (0 %, 7.5 %, 15 %, and 30 %) and the applied air pressure on the liquid surface (0.5 bar, 1 bar, and 2 bar). Cannabis plants were grown in a single 12 m^2 growth chamber at a density of six plants per m^2 for a total of 72 plants. Nine were used as a negative control (no infusion) and nine as a positive control (0 % sucrose), with three plants per applied pressure group. The remaining 54 plants were organized into groups of six in combinations of the two factors. Since each pressure vessel had limited reach due to the length of the infusion tubing, plants were distributed across three sections on the cultivation table. Each section contained all plants within the group at a single applied pressure. This resulted in 24 plants, which were randomly distributed per individual section.

2.2. Plant material and cultivation

One genotype of the variety Charlotte's Angel®, a chemical phenotype (chemotype) III with high total cannabidiol (CBD) levels and low total tetrahydrocannabinol (THC) levels (always below 1 %), was used in this study. Mother plants were cultivated in the greenhouse of the Department of Agronomy, University of Ljubljana, under a natural light regimen of high-pressure sodium (HPS) lamps with a photoperiod of 18 hours light and 6 hours dark (18 L: 6D) for vegetative growth. Temperature was maintained at 24°C/18°C and humidity between 50 % and 70 %. Plants were irrigated according to the Aptus (Aptus Plant Tech, Netherlands) nutrient schedule for vegetative growth.

Vegetative propagation of mother plants was begun on 9 November 2022 (day 0) in a greenhouse, following the method outlined by Cervantes (Cervantes, 2006). After 19 days, rooted clones were transplanted into 0.9 L pots filled with a perlite and soil mixture. After 18 days of vegetative growth, plants were topped and transferred to 7.5 L pots

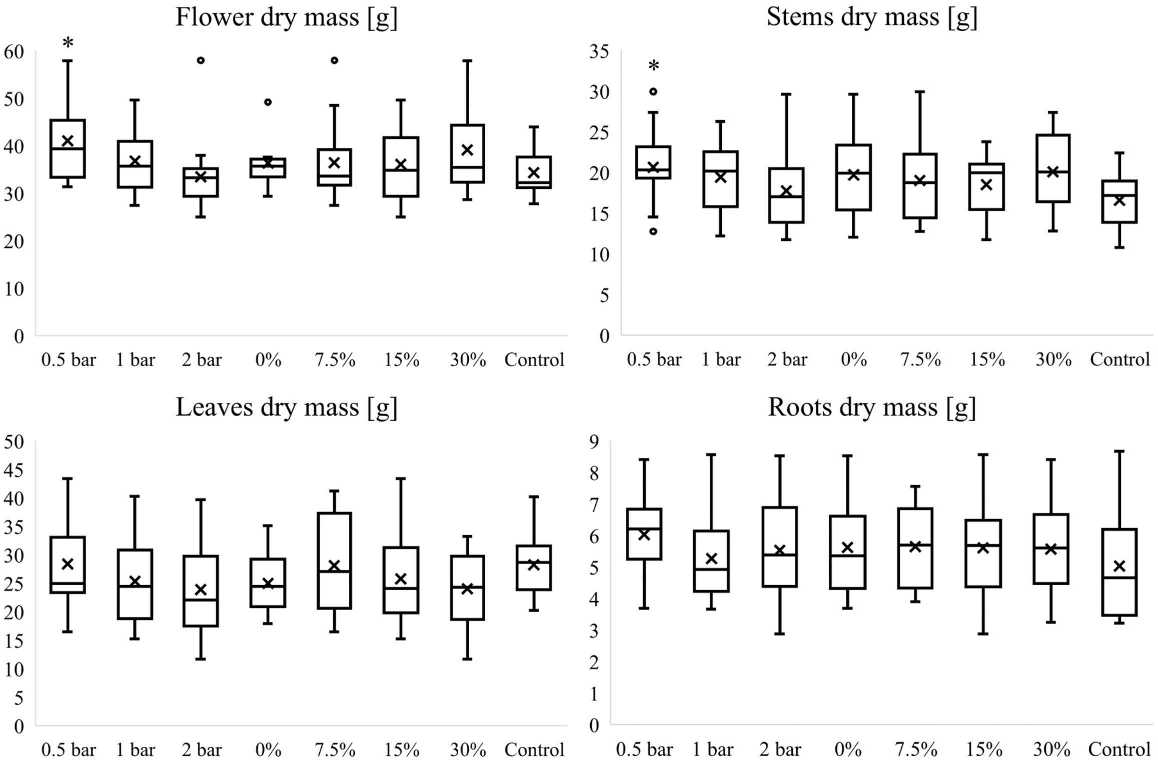


Fig. 5. Box plot comparisons of plant organ dry mass after harvest. Plants were grouped based on either applied pressure or sucrose concentration in the infusion solution. The negative control group contained nine plants, while each applied pressure group contained 21 plants, and each sucrose concentration group contained 18 plants, except the group with 0 % sucrose, which only contained nine plants. Each group was compared to the negative control group (no infusion). A two-way ANOVA with Tukey's post-hoc test was used to calculate statistical significance: (*$p < 0.1$, **$p < 0.05$, ***$p < 0.01$). All other morphological measurements showed no significant differences compared with negative control (raw data available in Supplementary Data 2).

containing the same substrate mixture (day 37). Vegetative growth continued for an additional 12 days in the greenhouse before plants were transferred to the controlled growth chamber (day 49) under artificial lighting (8 x Lumatek Pro 600 W GIB Lighting Pure Bloom HPS bulbs). The flowering phase commenced on 16 January 2023 (day 68), under a 12-hour light and 12-hour dark (12 L: 12D) cycle, with temperature and humidity set to 26°C/20°C and between 35 % and 50 %, respectively. On day 82, leaves and inflorescences from the lower one-third of the plants were removed. Plants were irrigated weekly following the Aptus Nutrient Schedule - PRO for flowering. Growth was terminated on 22 March 2023 (day 133), and plants were harvested for analysis.

2.3. Plant infusion protocol

The infusion solution was prepared using distilled water and the corresponding concentration of sucrose (0 %, 7.5 %, 15 % and 30 % w/v). The solution was poured into 800 mm-long polyvinyl chloride (PVC) tubes (internal diameter of 8 mm) that were positioned vertically on a stand, beside each infused plant. The bottom ends of these tubes were connected to standard tubing for intravenous (IV) infusion. On 18 January 2023 (day 70), each of the test plants was injected with a standard 20-gauge hypodermic needle (0.9 mm external diameter) attached to the end of the IV tubing (Fig. 1). Prior to the injection, some solution was run through, so no air bubbles were present. The needle was injected diagonally into the bottom-most nodium of each test plant (Fig. 2**a, b**). Given the relatively small stem diameters at the time of injection (approximately 5 mm), we aimed to position the needle hole so that it extended from the hurd to the pith. This approach was based on findings in tree studies, which suggested that xylem tissue was the most effective conduit for the transport of exogenous carbohydrates (Zhang et al., 2023).

After all plants were injected, the tops of the PVC tubes were connected to a compressor, and the corresponding pressure (0.5 bar, 1 bar, and 2 bar) was applied, and the solution levels were marked on the PVC tubes. The system remained pressurized for the duration of the experiment. To further ensure proper placement, we monitored the flow rate of the medium after each injection. If there was no immediate flow, we slightly adjusted the needle position until a steady flow was achieved. This served as a practical indicator of successful placement within a functional transport route.

2.4. Infusion flow measurements

The injection volumes were determined by measuring the change in the height of the solution column in a PVC tube multiplied by the internal column intersection surface. Interpolation was used to estimate measurements on days when direct data were unavailable.

2.5. Morphological measurements

Morphological measurements were conducted on days 78 and 133 (growth termination day), including plant height (excluding roots), main stem diameter, number of lateral branches, length of lateral branches, number of nodes on the main lateral branch, and main lateral branch diameter. After harvest (day 133), plants were divided into separate organs (flowers, stems, leaves, roots) and dried for 112 hours at 40°C, followed by an additional 48 hours at 75°C. After drying, the organs of each plant were weighed separately.

2.6. Physiological measurements

Physiological measurements were performed twice during the growth period (days 78–79 and 113–114) using the LI-6400XT (LI-COR

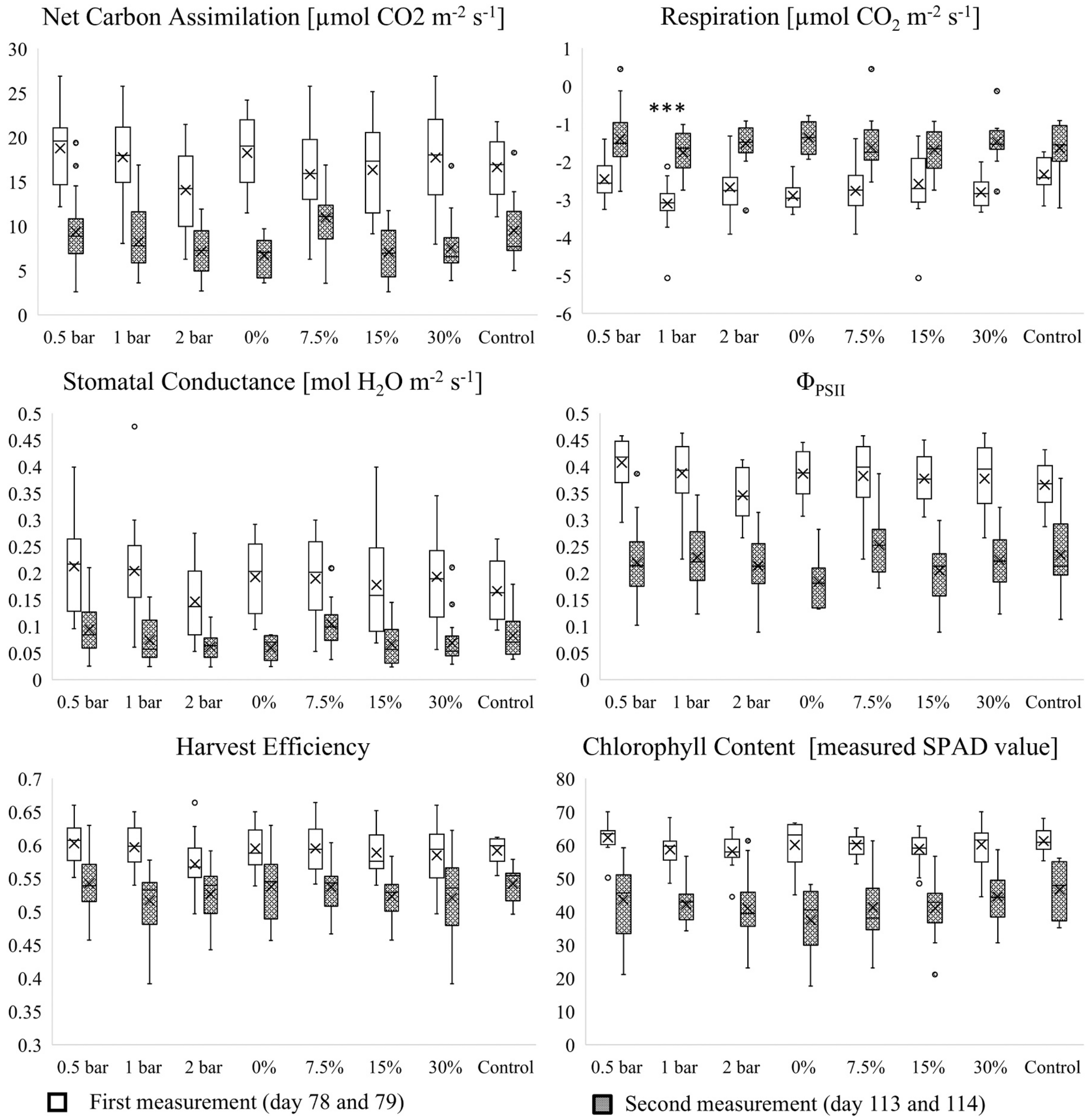


Fig. 6. Box plot comparisons of the chosen physiological parameters. Plants were grouped based on either applied pressure or sucrose concentration in the infusion solution and compared to the negative control group (no infusion). The negative control group contained nine plants, while each applied pressure group contained 21 plants, and each sucrose concentration group contained 18 plants, except the group with 0 % sucrose, which only contained nine plants. Each group was measured in two sessions, first on days 78 and 79 and second on days 113 and 114 of the growth period. A two-way ANOVA with Tukey's post-hoc test was used to calculate significance: $*p < 0.1$, $**p < 0.05$, $***p < 0.01$. Circles represent outliers.

Biosystems), with flow set at 300 μmol s^{-1}, CO_2 reference at 400 μmol s^{-1}, photosynthetically active radiation (PAR) at 1000 μmol m^{-2} s^{-1}, and block temperature at 26°C. On each plant, a well-developed and healthy leaf was selected and measurements were made with the LI-6400XT, firstly of PAR at 1000 μmol m^{-2} s^{-1} to measure net carbon assimilation (net photosynthesis), along with selected parameters: stomatal conductance, harvest efficiency (F_v'/F_m'), and the effective quantum yield (ϕ_{PSII}). Afterwards, the measurement was repeated on the same leaf at PAR set to 0 μmol m^{-2} s^{-1} to measure the respiration of the leaf. Chlorophyll content was measured using the Soil Plant Analysis Development SPAD-502Plus chlorophyll meter, (Konica Minolta). SPAD measurements were also taken two times during the growth period on days 78 and 114.

2.7. Cannabinoid identification and quantitation

For sample preparation, the topmost inflorescence of each plant was harvested at the conclusion of the experiment (day 133) and dried along with the other plant tissues. After drying, the inflorescences were ground using a coffee grinder, and 500 mg of the ground sample was suspended in 50 mL of methanol at room temperature. The suspension was vortexed for two minutes, followed by filtration through a 0.22 μm polytetrafluoroethylene (PTFE) syringe filter.

For high performance liquid chromatography (HPLC), 20 μL of the filtered solution was diluted with 980 μL of methanol in an HPLC vial, and 5 μL of this diluted solution was injected into the HPLC system (Knauer Smartline) equipped with a CORTECS Shield RP18 column (p/n: 186008685) heated to 35.0°C and an autosampler (Knauer Smartline Midas 3900). The mobile phase was mixed according to manufacturer's instructions and contained 59 % acetonitrile and 41 % distilled water

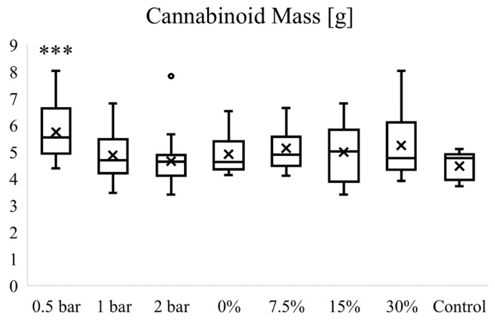


Fig. 7. Box plot comparison of total cannabinoid yield per plant. Plants were grouped based on either applied pressure or sucrose concentration in the infusion solution. The negative control group contained nine plants, while each applied pressure group contained 21 plants, and each sucrose concentration group contained 18 plants, except the group with 0 % sucrose, which only contained nine plants. Each group was compared to the negative control group (no infusion). A two-way ANOVA with Tukey's post-hoc test was used to calculate statistical significance: (*$p < 0.1$, **$p < 0.05$, ***$p < 0.01$). Circles represent outliers.

with 0.1 % trifluoroacetic acid, all HPLC grade. The flow rate was set at 2 mL/min with an isocratic method (no gradient) for a total runtime of 26 minutes per sample. The detector used was a Knauer Smartline 2600 UV detector at 228 nm and peaks were identified by comparison with known standards. Final concentrations were calculated based on standard curves for 16 cannabinoids using a combination of two standard solutions of nine neutral cannabinoid forms and seven acidic forms (neutral cannabinoid standards, Restek No. 34132, and acidic cannabinoid standards, Restek No. 34144).

2.8. *Data analysis and tests of statistical significance*

Fold change compared with negative control (plants with no infusion) was calculated for each measurement to assess the impact of the two factors (applied pressure and sucrose concentration). A two-way ANOVA with Tukey's post-hoc analysis was performed to determine the statistical significance of the different applied pressures and sucrose concentrations. No statistically significant interactions were observed between applied pressure and sucrose concentration for all dependent variables.

The focus was placed only on the comparison of all groups to the negative control. Additionally, Dunnett tests were conducted to assess the significance of individual combinations of factors compared to the negative control group.

All statistical tests were performed using the R software, version 4.3.2 (2023–10–31 ucrt). All data used in this study are available as supplementary information (Supplementary Data 1 and 2).

3. Results

3.1. *Injected volume of infusion solution*

The injected volumes were calculated for each test plant and subsequently averaged across different sucrose concentrations and applied pressures (Fig. 3**a, b**). The system pressure had the most significant impact on the total injected volume, with higher pressures resulting in greater injected volumes (raw data available in Supplementary Data 1). Although sucrose concentration had a less pronounced effect, it was observed that the 0 % sucrose solution (distilled water) resulted in higher injected volumes than the sucrose solutions because of its lower viscosity.

3.2. *Comparison of morphological measurements of plant groups*

Groups with infused plants showed, on average, greater mass and height at harvest compared to the control (Fig. 4**a, b**). However, only the plants injected at an applied pressure of 0.5 bar showed a significant increase in height.

Next, dry masses of plant organs were compared among the groups of applied pressure and sucrose concentration (Fig. 5). Although all groups had, on average, higher dry masses of flowers, stems and roots than the control group, only the group with an applied pressure of 0.5 bar showed a significant increase in flower and stem dry mass compared with control. Interestingly, all test groups had a lower leaf biomass compared to the negative control, but the differences were not statistically significant.

3.3. *Comparison of physiological measurements of plant groups*

To evaluate the physiological responses of the studied plants, we selected six parameters: net carbon assimilation, respiration, stomatal conductance, the effective quantum yield (ϕ_{PSII}, harvest efficiency (F_v'/F_m'), and chlorophyll content. Among these, only respiration between plants under 1 bar of applied pressure and control plants on day 78 showed a statistically significant difference ($p < 0.01$), with a fold change of 1.33. The other comparisons with the control group had p values above the 0.1 significance threshold (Fig. 6).

3.4. *Determination of cannabinoid yield*

Statistical analysis showed that the cannabinoid yield per plant was significantly higher in treatments with an applied pressure of 0.5 bar and 15 % (6.00 g/plant) and 30 % sucrose (5.99 g/plant) compared to control plants without infusion (4.48 g/plant). At an applied pressure of 0.5 bar, the cannabinoid yield per plant increased with the increase in sucrose concentration, while this was not observed at either of the higher pressures. In fact, a decrease in cannabinoid yield per plant was observed in treatments at 2 bar of applied pressure and 0 % (4.46 g/plant) and 15 % sucrose (4.21 g/plant) compared with the negative control. In general, 0.5 bar produced the highest cannabinoid yield, while the highest pressure resulted in the lowest yield (Fig. 7). The cannabinoid with the highest concentration (from 10.72 % to 12.42 %) was CBDA, with no statistical difference between treatments. The lowest total THC concentration was found in the control plants (0.36 %) and the highest at 0.5 bar and 7.5 % sucrose concentration (0.43 %) (Fig. 8).

3.5. *Effects of PSIS on yield factors*

Our results indicate that an applied pressure of 0.5 bar combined with sucrose concentrations of 15 % and 30 % had the most substantial positive impact on two of the three yield factors (flower dry mass and cannabinoid yield). Notably, the increases in flower dry mass and cannabinoid yield were statistically significant, with improvements ranging between 31 % and 34 % in comparison to the negative control (Fig. 9).

4. Discussion

This study aimed to evaluate the effects of PSIS in cannabis plants on morphology and physiology, as well as to assess its potential impact on the three main yield factors (total plant dry mass, flower dry mass, and cannabinoid yield). All treated cannabis plants were successfully infused with a sucrose solutions, and the infusion device, as initially described by Boyle (Boyle et al., 1991), was modified to allow more precise control over the applied pressure and to more accurately measure flow rates. Consistent with previous studies, it was found that the magnitude of applied pressure significantly affected the volume of solution injected, with distilled water (0 % sucrose) flowing with less resistance than

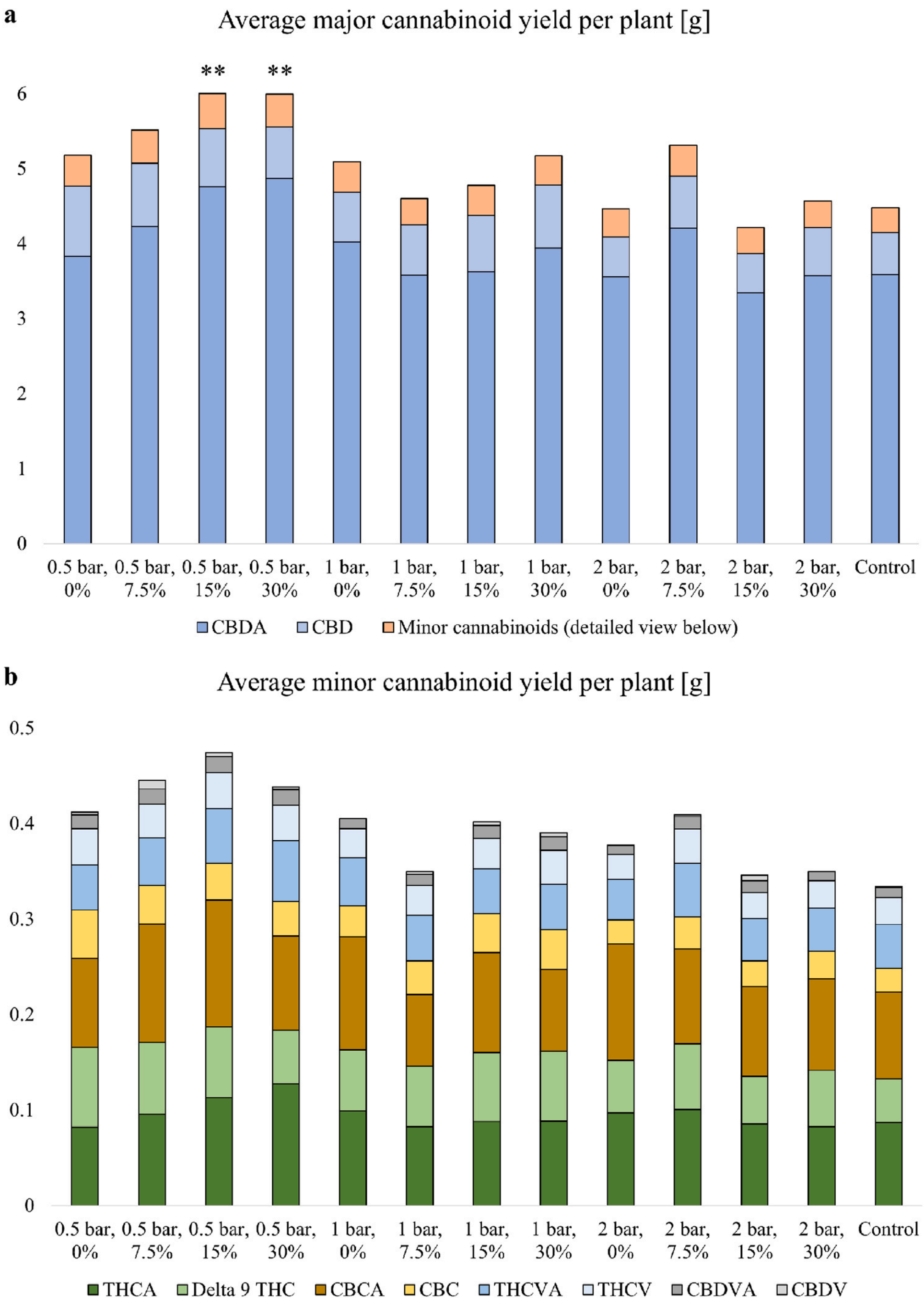
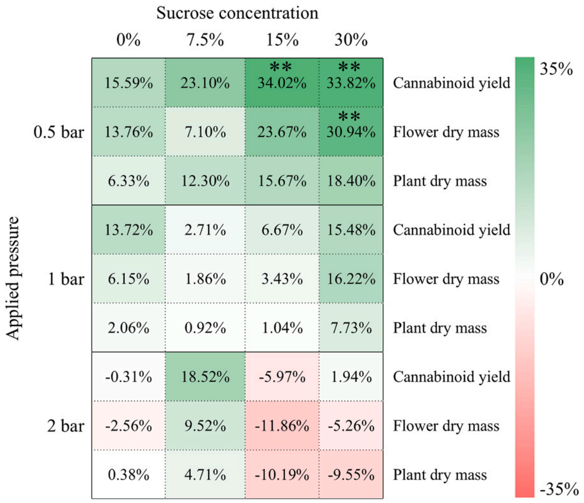


Fig. 8. Average cannabinoid yield with different ratios between a) major and b) minor cannabinoids for each combination of the two factors, applied pressure and sucrose concentration. The negative control group contained nine plants, while each applied pressure group contained 21 plants, and each sucrose concentration group contained 18 plants, except the group with 0 % sucrose, which only contained nine plants. A two-way ANOVA with Dunnett's post-hoc test was used to calculate statistical significance compared to the negative control group: *$p < 0.1$, * *$p < 0.05$, * **$p < 0.01$.

sucrose solutions, although minimal variation was observed among the latter concentrations. These findings align with the observations reported by Zhou et al. (Zhou et al., 1997; Zhou and Smith, 1996). Viscosity affects the resistance that a fluid experiences while flowing through a capillary system, injection device, or the vascular systems of plants (Lucas et al., 2013). The viscosity of solutions increases with the concentration of dissolved substances; thus, sucrose solutions have a higher viscosity than distilled water and show higher flow resistance at the same pressure (Courel et al., 2000). Solutions with higher sucrose concentrations also exhibit different rheological properties and higher osmotic pressure. This pressure counteracts the applied pressure and reduces the rate at which a higher-concentration sucrose solution can be delivered. (Lech et al., 2018).

Our study also revealed that PSIS can alter plant morphology. Treated plants exhibited increased overall height and mass and produced a higher flower yield compared to the untreated control. Interestingly, the treated plants also showed a reduction in dry leaf biomass and lower chlorophyll content compared to controls. While these differences were not statistically significant, they are consistent with previous findings that introducing exogenous sucrose via PSIS inhibited chloroplast activity and photosynthesis (Abdin et al., 1998; Khan at al., 2016; Zhou et al., 1997; Zhou et al., 1999). We have made similar observations with net carbon assimilation, which was reduced, though not significantly, in plants injected with 15 % and 30 % sucrose solutions



Applied pressure		0%	7.5%	15%	30%
0.5 bar	Cannabinoid yield	15.59%	23.10%	** 34.02%	** 33.82%
	Flower dry mass	13.76%	7.10%	23.67%	** 30.94%
	Plant dry mass	6.33%	12.30%	15.67%	18.40%
1 bar	Cannabinoid yield	13.72%	2.71%	6.67%	15.48%
	Flower dry mass	6.15%	1.86%	3.43%	16.22%
	Plant dry mass	2.06%	0.92%	1.04%	7.73%
2 bar	Cannabinoid yield	-0.31%	18.52%	-5.97%	1.94%
	Flower dry mass	-2.56%	9.52%	-11.86%	-5.26%
	Plant dry mass	0.38%	4.71%	-10.19%	-9.55%

Fig. 9. Heatmap of the effects of applied pressure and sucrose concentration on the three yield factors (total plant dry mass, flower dry mass, and cannabinoid yield). The numbers represent the average percent change compared to the negative control group. A two-way ANOVA with Dunnett's post-hoc test was used to calculate statistical significance: $*p < 0.1$, $* *p < 0.05$, $* **p < 0.01$).

compared to the negative control on the second measurement day.

The only statistically significant influence of PSIS on the plants' physiological properties turned out to be an increase in respiration on the first measurement day under 1 bar of PSIS. Under injection pressure, the plants received more sucrose, which caused an increase in respiration, a phenomenon that has been observed before (Högberg and Ekblad, 1996; Monteiro et al., 2002).

Sucrose plays a crucial role in regulating secondary metabolism in plants, acting not only as an energy source but also as a signaling molecule (Horacio and Martinez-Noel, 2013). Sucrose influences the synthesis of secondary metabolites such as flavonoids, phenolic acids, and anthocyanins, which often accumulate in plants in response to stress conditions (Van den Ende and El-Esawe, 2014). For example, in *Melissa officinalis*, the presence of sucrose upregulated flavonoid biosynthesis via the phenylpropanoid pathway (Kim et al., 2020). In plants like *Angelica gigas* (Xu et al., 2009), *Panax quinquefolium* (Kochan et al., 2014), *Scutellaria baicalensis* (Park et al., 2016), and *Picrorhiza kurroa* (Verma et al., 2015), sucrose has been found to stimulate the production of secondary metabolites such as pyranocoumarins, ginsenosides, baicalein, and iridoid glycosides. Cannabinoids are the most important secondary metabolites in cannabis, and PSIS significantly elevated cannabinoid yield in this study, with the most substantial increases observed under conditions of low applied pressure (0.5 bar) and high sucrose concentration (15–30 %). Conversely, high pressures were associated with a decrease in productivity, suggesting that excessive applied pressure may induce adverse effects in cannabis plants.

Studies have shown that the amount and type of accumulated secondary metabolites vary depending on sucrose concentration. For instance, at higher sucrose levels, increased accumulation of anthocyanins and rutin has been observed in plants like *Fagopyrum esculentum* (Li et al., 2011). In *Rhaponticum carthamoides*, a 3 % sucrose concentration was optimal for the simultaneous accumulation of caffeoylquinic acid derivatives and flavonoids, highlighting the need for fine-tuning sucrose concentration to achieve optimal yields of plant material and bioactive compounds. On the other hand, sucrose concentrations above 7 % began to inhibit root biomass accumulation, likely due to induced osmotic stress and/or carbohydrate toxicity at higher concentrations (Skała et al., 2022). Our study showed that sucrose concentration alone had neither a positive nor a negative effect on the dry mass of flowers, cannabinoid yield, or total plant dry mass. This suggests that the lowest or highest threshold for sucrose concentration was not reached in this study, which means that further research is needed to clarify the influence of sucrose concentration on cannabis production. However, the potential of PSIS technology, particularly for enhancing cannabinoid yield, where increases of up to 34 % compared to control were observed, is noteworthy and consistent with Aluko et al. (Aluko et al., 2021), who found that sucrose can increase plant yield through several mechanisms that affect both biomass accumulation and the production of specialized metabolites.

Despite decades of research on PSIS across various plant species, it has yet to achieve commercial adoption, likely due to the high costs associated with large-scale implementation. However, given that cannabis is one of the most valuable crops per gram of inflorescence biomass, even a modest increase in yield could justify the additional investment in this technology. For PSIS to become commercially viable in cannabis cultivation, several challenges must be addressed. These include developing a standardized and efficient injection system, improving ease of use, and minimizing labor requirements to enhance scalability. Additionally, a robust injection system would be necessary to reduce the risk of sucrose leakage and contamination. Using sterile needles and a sterilized medium would also remain essential in commercial applications to prevent fungal infections. Future research should focus on optimizing system design, improving nutrient delivery efficiency, and evaluating its applicability across different cannabis varieties, including high-THC commercial strains. Furthermore, long-term studies are needed to assess the effects of PSIS on plant physiology, secondary metabolite production, and overall cultivation sustainability.

These results underscore the great potential of this technology for the high-yield cultivation of cannabis with higher levels of cannabinoids. For future studies, experiments to fine-tune low-pressure and high sucrose concentrations in the infusion solution should be conducted to further explore and validate the potential benefits of this innovative method of cannabis cultivation.

5. Conclusions

This study presents plant stem infusion of sucrose (PSIS) as a promising method for optimizing cannabis cultivation by enhancing both morphological and physiological traits and significantly increasing cannabinoid yields. Low infusion pressures (0.5 bar) and high sucrose concentrations (15–30 %) led to the most substantial improvements in flower production and cannabinoid yield. However, higher pressures (1–2 bar) were detrimental to these outcomes, indicating the importance of controlling infusion parameters to avoid stress-induced damage.

Overall, PSIS represents a novel and effective approach to cannabis cultivation, with the potential to significantly improve yield and quality for both medicinal and recreational markets. Future research should aim to refine this technique by fine-tuning sucrose concentrations and pressures, as well as expanding the study to large plant populations of different genotypes and chemotypes to confirm these initial findings. This method holds great promise for growers seeking to optimize their production and meet the increasing demand for high-quality cannabis.

CRediT authorship contribution statement

Zupančič Klemen: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Zupančič Luka:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Lenarčič David:** Writing – review & editing, Supervision, Data curation. **Flajšman Marko:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Data curation. **Bitežnik Luka:** Writing – original draft, Methodology, Investigation, Data curation. **Malík Matěj:** Writing – review & editing, Writing – original draft, Investigation, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2025.120880.

Data availability

Data will be made available on request.

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