



Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)

<https://dergipark.org.tr/en/pub/yyutbd>



ISSN: 1308-7576

e-ISSN: 1308-7584

Research Article

Efficient *In Vitro* Micropropagation of Hemp (*Cannabis sativa*) Genotype

Cennet YAMAN^{*1}, Güngör YILMAZ², Talip KOÇER³

^{1,2,3}Yozgat Bozok University, Faculty of Agriculture, Department of Field Crops, Yozgat, Türkiye

¹<https://orcid.org/0000-0002-2364-8171>, ²<https://orcid.org/0000-0003-0070-5484>, ³<https://orcid.org/0000-0002-2434-8314>

* Corresponding author: cennet.yaman@bozok.edu.tr

Article Info

Received: 16.10.2025

Accepted: 10.04.2026

Online published: 01.06.2026

DOI: 10.29133/yyutbd.1804815

Keywords

Cannabis sativa,
Explants,
Growth regulators,
Micropropagation,
Shoot regeneration

Abstract: In this study, an effective regeneration and micropropagation protocol was developed from segregating the F₄ industrial hemp genotype Narlısaray. The apical shoot meristem and nodal (from different parts of the plant) explants were cultured at various concentrations of TDZ, BAP or Kin. Shoot regeneration was observed in all treatments, including the control treatment. The maximum number of shoots per explant (4.7) was obtained at 1.0 mg l⁻¹ TDZ, and was statistically different from 0.5 mg l⁻¹ (4.3) and 2.0 mg l⁻¹ TDZ (4.0). The longest shoots (6.5 cm) were recorded on 1.0 mg l⁻¹ TDZ. The position of the nodal explant source on the plant also affected its regeneration ability. The 1st stem node explant was more responsive to *in vitro* regeneration than the 2nd stem node. All tested concentrations of IBA induced root regeneration, with frequencies ranging from 62.5% to 87.5%. The regenerated plants were successfully acclimatized, reaching a maximum survival rate of 66.7%. This propagation technique can contribute to future hemp rapid breeding programs.

To Cite: Yaman, C, Yılmaz, G, Koçer, T, 2026. Efficient *In Vitro* Micropropagation of Hemp (*Cannabis sativa*) Genotype. *Yuzuncu Yil University Journal of Agricultural Sciences*, (1): 1804815. DOI: <https://doi.org/10.29133/yyutbd.1804815>

1. Introduction

The hemp plant, *Cannabis sativa* Linn (Cannabaceae family), is a high-yielding, annual industrial crop plant grown primarily for obtaining fiber, and obtaining biomaterial in tissue engineering, and as a source of obtaining drugs (THC, CBD, and other phytocannabinoids, etc.) in the pharmaceutical industry (Andre et al., 2016), extraction of oil from the seeds, and more than 25000 bioproducts (Crini et al., 2020; Abbaszadeh et al., 2026).

Its cultivation was legally restricted in many countries due to the psychoactive effect of one of its compounds, Δ -9- tetrahydrocannabinol (THC), for a long time (Liu et al., 2022). These legal restrictions have significantly reduced and slowed down the research and development activities on *C. sativa* globally. Recently, the Turkish government has eased restrictions on hemp cultivation due to its multipurpose use across sectors, including industrial (fiber production), food (seed and oil), and pharmaceutical applications. The permissive legal threshold level of THC in dried flowering plant parts of industrial hemp is 0.1 % in Australian states, Columbia, Mexico, Switzerland, and Uruguay. Whereas, this percentage is 0.2% in Europe and 0.3% in Brazil, Canada, China, and the USA (Adhikary et al., 2021).

The cultivation of this economically valuable plant is increasing day by day. Traditionally, the fiber type (commonly called hemp, with Δ^9 -THC < 0.3%) of *C. sativa* is predominantly monoecious (hermaphrodite), mostly cultivated by seeds as this characteristic produces more uniform material and

its cultivation has been highly mechanized (Chandra et al., 2017). In contrast, drug type (commonly called cannabis, with Δ^9 -THC > 0.3%) of *C. sativa* is often propagated using clonal methods (Campbell et al., 2019; Talei et al., 2025) to reduce the high levels of phenotypic diversity exhibited in seedling populations and to produce high-quality, genetically and phenotypically uniform crops that meet consumer preferences and comply with government regulations (Lata et al., 2010). Although more expensive than seed, this approach is efficiently used by some *C. sativa* producers to commercially produce a more uniform crop containing CBD and other non-psychoactive cannabinoids (Ioannidis et al., 2020; González et al., 2025). Also, many scientists are developing new varieties to serve this purpose, given the economic importance of *C. sativa*. The predominantly annual dioecious nature of *C. sativa* is an important obstacle to slowing down traditional breeding efforts (Clarke et al., 2016). Therefore, clonal multiple propagation practices offer a valuable tool for producing uniform male and female individuals for the breeding program of *C. sativa*.

Clonal multiple propagations from the male or female plants are either generally produced through stem cuttings of sub-leaf microsprouts from a mother plant, which is vegetative production under greenhouse conditions, or accomplished by tissue culture methods (Adhikary et al., 2021). Indoor *C. sativa* culture via stem cuttings can produce large numbers of genetically similar plants, but they become less vigorous over time. That is, mother plants can become susceptible to insect, bacterial, fungal, and viral diseases that may come from the environment, and therefore their cuttings may harbor unwanted diseases (Monthony et al., 2021). On the contrary, micropropagation by tissue culture is an effective method for the mass propagation of disease-free plants in a highly controlled environment, using aseptic techniques, in less time, and has been widely used for many commercial plants for a long time (Shekhawat et al., 2015; Wongchaya et al., 2025).

In recent years, numerous micropropagation studies have been conducted on *C. sativa*, and various protocols have been proposed (Lata et al., 2017; Page et al., 2020; Ioannidis et al., 2020; Holmes et al., 2021; Monthony et al., 2021; Wróbel et al., 2022). However, despite the availability of these protocols, *C. sativa* exhibits a complex genetic architecture and inheritance pattern, as it is predominantly dioecious and obligately outcrossing. This genetic complexity leads to high phenotypic variability, variation in sex expression, and pronounced polymorphism, contributing to its considerable biological plasticity (Razumova et al., 2016; Hurgobin et al., 2021; Ioannidis et al., 2022; Ingvarsdén and Brinch-Pedersen, 2023). Consequently, as studies continue to reveal the genetic and phytochemical diversity of *C. sativa*, many researchers emphasize the need to develop genotype-specific regeneration protocols (Chaohua et al., 2016; Holmes et al., 2021; Monthony et al., 2021). Therefore, in this study, the effects of some cytokinins and explant sources on micropropagation of Narlısaray, a native population genotype of Türkiye, were determined.

2. Materials and Methods

2.1. Plant material

The seeds of the advanced segregating F₄ breeding line Narlısaray of Hemp (hereafter called Narlısaray), were obtained from the Hemp Research Institute Yozgat Bozok University, Türkiye.

2.2. Surface disinfection and germination conditions

The seeds were soaked in 70% ethanol for approximately 1 minute and then surface disinfected by shaking in a solution containing 0.5% NaOCl (15% v/v commercial bleach) and 0.1% Tween 20 (v/v) for 20 minutes. The sterilized seeds were rinsed 3 × 5 min in sterile distilled water (Lata et al., 2016).

The sterilized seeds were placed on a germination medium containing 1× Murashige and Skoog (1962) MS medium supplemented with 3% sucrose (Duchefa). The pH of the medium was adjusted to 5.7±0.1 with 0.1N NaOH or 0.1 N HCl before the addition of 0.6% (w/v) plant agar (Duchefa, The Netherlands). The seeds were maintained in a growth chamber at room temperature (25 ± 1 °C) under a 16/8 h light/dark photoperiod, 60% relative humidity, and cool white fluorescent light (PHILIPS 36 W/830 G13 1214 mm lamps, intensity of 40 μmol m⁻²s⁻¹ PPFD) for 15 days to germinate.

2.3. *In vitro* shoot proliferation

Different experiments on *in vitro* hemp propagation were conducted using apical shoot meristems and 1st and 2nd stem node explants. Narlisaray phyllotaxy is characterized by an opposite leaf arrangement with only two leaves and two axillary buds at each node (Figure 1). Therefore, the seeds were germinated *in vitro* to obtain appropriate explants. After 3 weeks of germination, all three explants were cultured for 2 weeks on MS medium without PGRs to achieve vegetative maturity. The 1st, 2nd node and apical shoot meristem explants (as indicated in Figure 1) were used as explants. The explants were transferred into Magenta™ vessels and the cultures were maintained in a growth chamber.



Figure 1. The position of apical shoot meristem, 1st stem node and 2nd stem node on *in vitro* germinated seedlings of *C. sativa*.

In the first experiment, MS medium supplemented with 0, 0.5, 1.0, 2.0 or 4.0 mg l⁻¹ concentrations of TDZ, BAP or Kin were tested to induce morphogenesis using the apical shoot meristem. In the second experiment, the experiment was continued by culturing the 1st and 2nd node explants on MS medium supplemented with 0.2, 0.5, 1.0, or 2.0 mg l⁻¹ of TDZ (Figure 1) to induce shoots.

A total number of 16 explants were used in each treatment. These were distributed equally across 4 replications (+ culture vessels) for each PGR treatment. All explants were cultured in a growth chamber at room temperature (25 ± 1 °C) under a 16/8 h light/dark photoperiod, 60% relative humidity, and cool white fluorescent light (PHILIPS 36 W/830 G13 1214 mm lamps, 40 µmol m⁻²s⁻¹ PPFD). After 15 days of culture, the following parameters were recorded for the first experiment: Shoot induction (%), callus induction (%), number of shoots per explant, number of node per explant, shoot length (cm), and for the second experiment: Shoot induction (%), callus induction (%), shoot length (cm), number of shoot, number of node formation per explant, number of leaf formation per explant, leaf length (cm).

2.4. Rooting of shoots from nodal explants

All of the regenerated shoots in the experiment were subjected to rooting. on ½ × MS medium containing 0, 0.2, 0.5, 1.0, or 2.0 mg l⁻¹ of IBA, to determine the optimum medium for rooting. The experimental data about rooting (%), number of roots per explant, and root length (cm) was recorded after two weeks (14 d) of culture.

2.5. Acclimatization

All rooted plantlets were acclimatized. They were carefully removed from the vessels and washed under running tap water to remove agar. They were transferred to 50 Cell Seedling Trays (140 cc) using a sterilized perlite: peat (1:1) mixture and acclimatized in a climate chamber with 60% humidity under cold white fluorescent lights (16:8 h-l light/dark photoperiod, 36 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PPFD). A transparent dome cover with two vents was placed over the tray to prevent excessive moisture loss through transpiration. The plants were gradually acclimated and hardened (induced vegetative maturity) for two weeks. Thereafter, the percentage and number of successfully acclimatized plantlets were carefully counted.

2.6. Statistical analysis

All data on growth parameters and acclimatization percentage (%) were analyzed using one-way ANOVA (IBM SPSS 25), followed by Duncan's multiple-range test ($P \leq 0.05$) to compare treatment means. The percentage values were arcsin transformed by before statistical analysis. The standard errors of all treatment means were calculated and given as $\pm$ next to their mean values.

3. Results and Discussion

Micropropagation is a plant tissue culture practice that represents an alternative and promising approach for large-scale propagation of genetically uniform *C. sativa* plants with high phytosanitary quality, which can be produced indoors (Adhikary et al., 2021; Page et al., 2021).

This study provided a safe protocol for *in vitro* production, rooting, and acclimation of *C. sativa*. Apical shoot-tip and nodal explants were preferred under *in vitro* replication studies of *C. sativa*. These explants have been selected because they contain meristematic regions (Kose et al., 2021; Chandola et al., 2023) or have the capacity to induce meristems during culture. The 1st and 2nd stem node explants were appropriate explants for micropropagation and preservation of germplasm through direct organogenesis, but they are not common explants for genetic transformation (Lata et al., 2009; Wang et al., 2009; Lata et al., 2016; Smýkalová et al., 2019; Monthony et al., 2021; Page et al., 2021; Wróbel et al., 2022). However, the micropropagation method using apical shoot meristem and 1st and 2nd stem node explants in this study is a method of lower risk of genetic instability, as reported by Rao and Lee (1986). Therefore, in this study, the effect of cytokines was first determined using apical shoot explants, and then the performance of nodal parts (1st and 2nd stem node explants), which are important for micropropagation, was examined in the medium containing the cytokine concentration that yielded the best regeneration.

3.1. Effect of apical-shoot meristem explant on shoot induction under different PGRs

The diversity and concentration of plant growth regulators in a culture medium significantly affect the regeneration capacity of explants in tissue culture techniques (Gehl et al., 2020). Therefore, the apical shoot-tip explants were cultured for multiple shoot induction to optimize the best concentration of cytokinin (TDZ, BAP, or Kin) for shoot induction (Table 1). The explants exhibited friable callus throughout culture, without regeneration of organogenic or somatic embryogenic structures. Another frequently observed result was the growth of the main shoot, with suppression of lateral shoots and no possibility of shoot proliferation (apical dominance). *C. sativa* exhibited strong apical dominance, which is problematic during *in vitro* culture. This behavior is attributed to hormonal imbalances that lead to the formation of callus (Smýkalová et al., 2019).

Table 1. Effect of three different cytokines (TDZ, BAP, Kin) on *in vitro* plant growth parameters of *C. sativa*

PGRs (mg l ⁻¹)	Co nc.	Shoot induction (%)	Callus induction (%)	Number of shoots per explant	Number of nodes per explant	Shoot length (cm)
Control	0.0	100.0	33.3±4.2 ^c	1.4±0.4 ^{cd}	2.2±0.2 ^{cdef}	3.6±0.3 ^d
	0.5	100.0	91.7±0.0 ^{ab}	4.3±0.4 ^{ab}	2.3±0.1 ^{bcd}	5.9±0.1 ^b
TDZ	1.0	100.0	100.0±0.0 ^a	4.7±0.5 ^a	2.8±0.1 ^{ab}	6.5±0.3 ^a
	2.0	100.0	100.0±0.0 ^a	4.0±0.0 ^{ab}	1.9±0.1 ^{efg}	4.5±0.1 ^c
	4.0	100.0	100.0±0.0 ^a	3.0±0.0 ^c	1.9±0.1 ^{efg}	2.1±0.2 ^{de}
	0.5	100.0	100.0±0.0 ^a	1.7±0.1 ^{ed}	2.0±0.1 ^{defg}	1.7±0.1 ^e
BAP	1.0	100.0	100.0±0.0 ^a	1.2±0.1 ^{ed}	2.6±0.1 ^{abc}	3.9±0.1 ^{cd}
	2.0	100.0	75.0±7.2 ^{abc}	3.5±0.4 ^{bc}	2.7±0.1 ^{ab}	2.6±0.1 ^e
	4.0	100.0	91.7±4.2 ^{ab}	2.0±0.3 ^d	1.5±0.1 ^g	1.6±0.2 ^e
	0.5	100.0	50.0±14.5 ^{ab}	1.0±0.0 ^e	3.0±0.1 ^a	3.5±0.1 ^d
Kin	1.0	100.0	66.7±8.4 ^{ab}	1.0±0.0 ^e	2.9±0.3 ^a	4.0±0.4 ^{cd}
	2.0	100.0	50.0±12.5 ^{ab}	1.3±0.3 ^{ed}	2.4±0.2 ^{bcd}	2.1±0.0 ^{de}
	4.0	100.0	50.0±14.5 ^{ab}	1.0±0.0 ^e	1.8±0.1 ^{fg}	2.0±0.3 ^e

Control: MS medium; Means followed by different letters are significantly different at p<0.05 according to Duncan's Multiple Range Test.

In this study, the results showed that the types and concentrations of PGRs had different effects on the growth parameters (Table 1). Shoot induction was noted regardless of the concentrations used in the experiment. Callus induction range 91.7-100% on MS medium enriched with different concentrations of TDZ. It was 75-100% on several concentrations of BAP. Inhibited callus induction of 50-66.7% was noted on all concentrations of Kin (Figure 2).

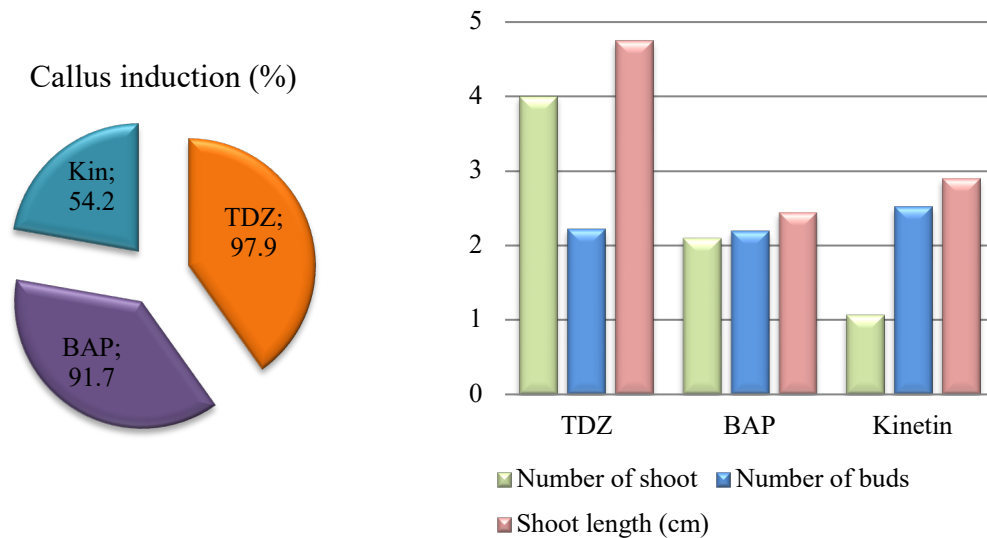


Figure 2. Effect of plant growth regulators on *in vitro* plant growth parameters of Narlisaray segregating population of *C. sativa*.

Number of shoots per explant ranged from 3.0-4.7, 1.2-3.5, and 1.0-1.3 on TDZ-, BAP-, and Kin-containing media, respectively, in the same order. The maximum number of shoots per plant (4.7) was obtained using 1.0 mg l⁻¹ TDZ (Table 1). Number of nodes per explant ranged from 1.9-2.8, 1.5-2.7, and 1.8-3.0 in TDZ-, BAP-, and Kin-containing media, respectively. The maximum number of nodes per explant was recorded at 0.5 mg l⁻¹ Kin, followed by 3.0 mg l⁻¹ Kin, and then shoot induction at 1.0 mg l⁻¹ Kin, 1.0 mg l⁻¹ TDZ, and 2.0 and 1.0 mg l⁻¹ BAP treatments, in the same order (which were statistically similar). The maximum shoot length of 6.5 cm was recorded at 1.0 mg l⁻¹ TDZ, followed by values of 5.9 cm at 0.5 mg l⁻¹ TDZ, which were statistically similar. MS medium modified with more or less than 1.0 mg/LBAP or Kin was either non-promotive or inhibitory (Table 1).

The findings showed that Kin increased the number of nodes, and TDZ influenced the number of shoots on the explants. These results confirm the studies of Lata et al. (2009) and Wróbel et al. (2022). In addition, Wang et al. (2009) found that TDZ was more effective than BA and Kin on the number of axillary shoots. Also, Stephen et al. (2023) reported that shoot and node numbers increased with two higher TDZ concentrations, but the shoots were shorter, and plant quality was lower. Inhibition of shoot elongation may result from the high cytokinin activity of TDZ (Huetteman and Preece, 1993). Unlike adenine-type cytokinins (BAP and kinetin), TDZ is more effective at low concentrations (Devi et al., 2011). TDZ has been reported not only to exhibit cytokinin-like activity but also, in some studies, to increase endogenous cytokine levels and contribute to breaking apical dominance by affecting auxin balance/transport (Dinani et al., 2018; Li et al., 2021). It is suggested that these effects may be more pronounced in some systems compared to BAP and Kin (Wang et al., 2025). Generally, 1.0 mg l⁻¹ TDZ could be considered to induce a positive effect on regeneration activities of hemp explants. In contrast to the present findings, Ioannidis et al. (2020) demonstrated that the application of 4.0 mg l⁻¹ TDZ in both full- and half-strength MS media was more effective in enhancing shoot number and shoot length in *C. sativa* than other tested concentrations, and further reported that BA was more effective than TDZ.

However, later, Lata et al. (2016) reported that mT (meta-topolin), a natural aromatic cytokinin, was superior to TDZ for shoot number and length in *C. sativa*. Monthony et al. (2021) and Page et al. (2021) emphasized in their studies that mT cytokinin promotes the formation of multiple shoots during *in vitro* propagation treatments of *C. sativa*. In contrast, Wróbel et al. (2022) reported that mT cytokinin had a lower stimulatory effect than BAP and TDZ on the shoot-forming explant ratio and the number of shoots per explant of *C. sativa*. Treatments of the same cytokinin can have varying effects on hemp genotypes belonging to several different species and in the same genus (Smýkalová et al., 2019; Holmes et al., 2021).

3.2. Effect of 1st and 2nd stem node explants on shoot induction

TDZ, containing regeneration medium induced variable shoots on the 1st and 2nd stem node explants (Figure 1). In the *in vitro* propagation study using hemp nodal explants, it was necessary to investigate the effect of explant position to determine the cause of variation observed during micropropagation, rooting, and acclimatization. Within the scope of this study, TDZ, a plant growth regulator that yields the best results under *in vitro* conditions, was used at 0.5, 1.0, and 2.0 mg l⁻¹ concentrations for the 1st- and 2nd-node explants (Table 2 and Figure 3). The effects of genotypes on *in vitro* micropropagation performance were investigated. Data were measured after 28 d of culture.

Table 2. Effect of TDZ and two different stem node explants (1st and 2nd) on *in vitro* plant growth parameters of *C. sativa*

TDZ (mg/)	Exp.	Shoot induction (%)	Callus induction (%)	Shoot length (cm)	Number of shoot	Number of node formation per explant	Number of leaves formed per explant	Leave length (cm)
0.5	1st	100	83.3±8.3 ^{ab}	3.94±0.0	2.83±0.1	3.75±0.0 ^a	8.50±0.0 ^a	1.51±0.0 ^{ab}
	2nd	100	58.3±8.3 ^b	2.40±0.2	2.50±0.3	2.42±0.2 ^b	7.17±0.4 ^{bc}	1.26±0.1 ^b
1.0	1st	100	66.7±8.3 ^b	2.34±0.0	3.33±0.1	2.67±0.1 ^b	7.30±0.4 ^{bc}	1.90±0.1 ^a
	2nd	100	58.3±8.3 ^b	2.10±0.1	3.25±0.1	2.33±0.2 ^b	6.53±0.4 ^c	1.57±0.3 ^{ab}
2.0	1st	100	100.0±0 ^a	2.83±0.3	3.25±0.5	2.67±0.2 ^b	7.67±0.2 ^b	1.70±0.1 ^{ab}
	2nd	100	83.3±8.3 ^{ab}	3.65±2.0	2.50±0.3	2.42±0.2 ^b	6.58±0.1 ^c	43±0.0 ^b

Control: MS medium; Means followed by different letters are significantly different at p<0.05 according to Duncan's Multiple Range Test.

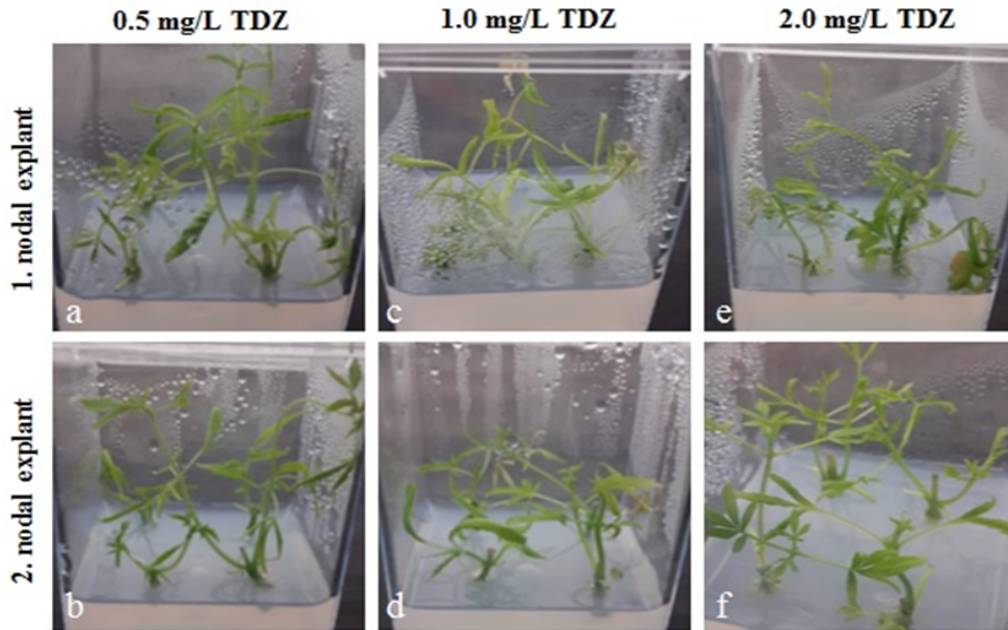


Figure 3. Effects of 0.5, 1.0, and 2.0 mg l⁻¹ TDZ on shoot induction from 1st- and 2nd-node explants, with explants facing upward.

The 1st- and 2nd-stem node explants with different TDZ concentrations responded differently to the analyzed parameters (Table 2). It was found that the 1st stem node was more successful at inducing shoots than the 2nd node explant type (Figure 4).

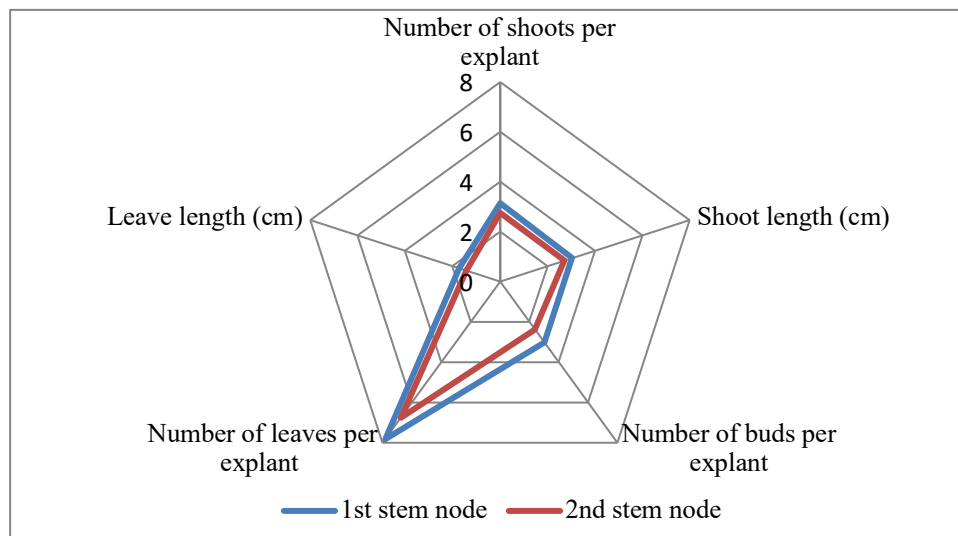


Figure 4. Effect of 1st and 2nd stem node explants upside-down on *in vitro* plant regeneration parameters of *C. sativa*.

Shoot induction was achieved with 100% success on all treatments. However, all TDZ concentrations induced callus before inducing shoots on both explants (Table 2). The 1st stem node explant exhibited the maximum callus induction in medium supplemented with 2.0 mg l⁻¹ TDZ (100%). Callus induction of the 1st stem node explant was more effective compared to the regeneration capacity of the 2nd node explant using any concentrations of TDZ (Figure 4).

All treatments were statistically similar for shoot length and number of shoots per plant, but showed significant differences in the number of nodes per plant, number of leaves per plant, and leaf length (Table 2). The 1st stem node explant exhibited the maximum number of nodes per plant (3.75)

and number of leaves per plant (8.50) on MS medium supplemented with 0.5 mg l⁻¹ TDZ. The maximum leave length (1.90 cm) was observed on the 1st stem node explant cultured on 1.0 mg l⁻¹ TDZ.

From studies on other species, it is known that long-term explant performance can be affected by various explant-related factors such as type (Monthony et al., 2021), season (Isah, 2020), developmental stage (Martini et al., 2022), and location within the plant (Marks and Myers, 1992). The difference in regeneration ability between nodal explants from the 1st and 2nd nodal regions of the plant in this study is an indication of this. In general, young explants offer many benefits in terms of growth viability and are more susceptible to micropropagation techniques than mature explants (Devi et al., 2011). However, in direct shoot regeneration from nodal parts on the same plant, the awakening of the axillary meristems in the nodal parts close to the root part is faster than that in other nodal parts located on the plant. Therefore, nodal explants from the lower parts form shoots faster and exhibit more stable morphology than those from the upper parts (Zhang et al., 2020). Shoots formed from nodal explants in the upper parts exhibit different morphological appearances and reduce sustainability in subculturing (Adamek et al., 2022). This is one of the most important reasons that negatively affect the production of plants with stable morphology in long-term subculturing of hemp (Page et al., 2021). Therefore, explant source can be considered one of the most important factors in rejuvenation treatments to overcome culture decline over time and reduce variation between treatments.

3.3. *In vitro* rooting

Rooting is frequently the most difficult stage in micropropagation (Holmes et al., 2021; Page et al., 2021) of medicinally important plants, which are known to produce high concentrations of phenolic acids and metabolites (Ranaweera et al., 2013), which induce oxidative stress and make it difficult to induce root meristematic tissues. IBA is a naturally occurring auxin and has been shown to stimulate root growth in *C. sativa* at 5 µM IBA (Lata et al., 2009), as verified in our investigation. *In vitro* rooting of *C. sativa*, raising the concentration of IBA in ½× MS medium, exhibited root induction without deterioration of root quality.

This study indicated no significant difference among the rooting techniques. The 2.0 mg l⁻¹ IBA treatment resulted in the best root induction (87.5%) and root number (4.8), but the 1.0 mg l⁻¹ IBA treatment favored the induction of the longest roots (2.2 cm) (Table 3 and Figure 5). Chaohua et al. (2016) reported that in the *in vitro* rooting treatment of *C. sativa*, approximately 80% of the shoots from ½ MS medium containing 0, 0.2, 0.5, 1.0 or 2.0 mg l⁻¹ IBA achieved good root development in the medium containing 0.5, 1.0 or 2.0 mg l⁻¹ IBA. Wróbel et al. (2022) examined the effect on rooting performance of *C. sativa* in ½ MS medium containing 0.5 mg l⁻¹ IAA and IBA, but found that there was no statistical difference between the analyzed parameters and, in parallel with our study, they recorded the rooting frequency as 66.7% and the number of roots as 2.2 in the IBA treatment. Movahedi et al. (2015) found IBA auxin to be more effective than NAA on rooting in *C. sativa*. No rooting was observed in the ½ MS medium, which was the control group, but the plants continued to grow.

Table 3. Effect of different concentrations of IBA on root induction

IBA (mg l ⁻¹)	Root induction (%)	Number of roots	Root length (cm)
0.0	0.0±0.0 ^b	0.0±0.0 ^b	0.0±0.0 ^c
0.2	62.5±12.5 ^a	3.4±0.9 ^a	0.4±0.1 ^{bc}
0.5	68.8±18.8 ^a	3.7±1.2 ^a	1.8±0.5 ^{ab}
1.0	81.3±6.3 ^a	4.2±0.7 ^a	2.2±0.8 ^a
2.0	87.5±7.2 ^a	4.8±0.6 ^a	0.4±0.2 ^{bc}

Control: MS medium; Means followed by different letters are significantly different at p<0.05 according to Duncan's Multiple Range Test.

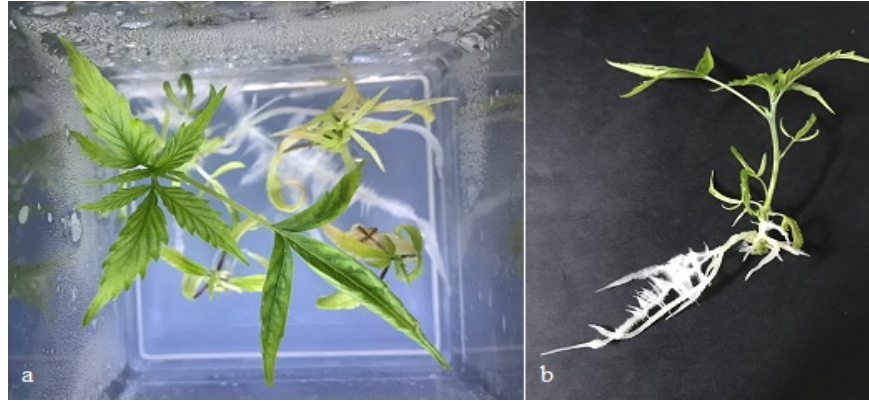


Figure 5. Rooting of *C. sativa* shoots in 1.0 mg l⁻¹ IBA medium (a, b).

Acclimatization: The final step in tissue culture propagation is the acclimatization of the plantlets. At this stage, the seedlings are removed from the jars/magenta and acclimated to the external environmental conditions. When removing plantlets from the medium, the roots should be carefully washed to prevent damage, and the agar washed to remove residual sucrose and prevent fungal growth. The present study's findings indicate that the maximum response to acclimatization was obtained from non-rooted seedlings in the control group (66.7%) (Table 4). The higher adaptation success of rootless seedlings obtained *in vitro* compared to rooted seedlings may be because *in vitro* roots are not fully functional anatomically and physiologically (Chandra et al., 2010). It has been reported that IBA-induced adventitious roots generally exhibit weak xylem-phloem connection, low conductivity, and limited lignification, which reduces water uptake efficiency under *ex vitro* conditions (Dev et al., 2019). Furthermore, it has been reported that root systems formed *in vitro* develop under high humidity and high-sugar conditions, leading to poor stomatal control, limited cuticle development, and increased susceptibility to transplantation shock (Mahendra et al., 2020; Dushimimana et al., 2022). In contrast, the fact that rootless shoots develop *in vitro* without experiencing rooting stress and achieve root formation under natural conditions during the adaptation process may have contributed to the development of a more functional root system and higher stress adaptation (Dimitrova and Nacheva, 2021; Grzelak et al., 2024; Thiem et al., 2023; Xiong et al., 2022). Murphy and Adelberg (2021) reported a proliferation technique in which apical and nodal parts are rooted in the external environment, while only shoot development occurs in the nutrient medium under *in vitro* conditions, and stated that it was a more economical method.

Table 4. Acclimatized ratio of *C. sativa* shoots from media containing several IBA concentrations

IBA (mg/L)	Acclimatization percentage (%)
0.0	66.7± 0.09 ^a
0.2	31.3±0.04 ^c
0.5	37.5±1.24 ^b
1.0	31.3±1.46 ^c
2.0	31.3±0.96 ^c

Control: MS medium; Means followed by different letters are significantly different at p<0.05 according to Duncan's Multiple Range Test.

4. Conclusion

This is the first successful report on micropropagation of plants from apical shoot meristems and nodal explants of segregating population of Narlısaray. This study highlighted the differential responses of various cytokinins. In particular, TDZ exhibited the best response for plant regeneration in Narlısaray. Additionally, 1st-stem-node explants exhibited greater shoot-regeneration potential. The need for breeding new varieties suitable for the hemp regeneration has high economic value. Increasing breeding opportunities through modern technologies will facilitate its breeding programs in many ways. The callus-forming and shoot-inducing abilities of this genotype can be evaluated in future secondary metabolite production studies under *in vitro* conditions.

Ethical Statement

Ethical approval is not required for this study because there was no specific type of research involving humans or animals.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Artificial Intelligence Declaration

The authors declare that no generative artificial intelligence tools were used at any stage of the preparation of this manuscript, including the writing, editing, or refinement of the text, or the creation of any images, figures, graphics, tables, or related titles.

Funding Statement

This study was supported by the Scientific Research Projects Coordination Unit (BAP) of Yozgat Bozok University under project number 6608-ZF/20-409.

Author Contributions

C.Y. literature search; writing; concept; project administration; design; resources; materials; data collection and/or processing; validation; analysis G.Y and T.K. interpretation; critical reviews; project administration and funding acquisition. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

We gratefully the Scientific Research Projects Coordination Unit (BAP) of Yozgat Bozok University for supporting our work as a project.

References

- Abbaszadeh, B., Valizadeh, N., Ağamirzaoğlu, M., & Bengisu, G. (2026). Responses of Different *Cannabis sativa* L. Populations to Plant Density and Various Fertilization Treatments. *Yuzuncu Yıl University Journal of Agricultural Sciences*, (1), 1697183.
- Adamek, K., Jones, A. M. P., & Torkamaneh, D. (2022). Accumulation of somatic mutations leads to genetic mosaicism in cannabis. *The Plant Genome*, 15(1), e20169.
- Adhikary, D., Kulkarni, M., El-Mezawy, A., Mobini, S., Elhiti, M., Gjuric, R., Ray, A., Polowick, P., Slaski, J. J., Jones, M. P., & Bhowmik, P. (2021). Medical cannabis and industrial hemp tissue culture: Present status and future potential. *Frontiers in Plant Science*, 12, 275.
- Andre, C. M., Hausman, J.-F., & Guerriero, G. (2016). *Cannabis sativa*: The plant of the thousand and one molecules. *Frontiers in Plant Science*, 7, 19.
- Campbell, L. G., Naraine, S. G., & Dufresne, J. (2019). Phenotypic plasticity influences the success of clonal propagation in industrial pharmaceutical *Cannabis sativa*. *PLOS ONE*, 14(3), e0213434.
- Chandola, V., Chandra, S., Nautiyal, A. R., Kumar, R. R., & Purohit, V. K. (2023). TDZ and zeatin induced in vitro shooting for efficient vegetative propagation in *Trillium govanianum*, an endangered alpine medicinal herb. *Vegetos*, 1–9.
- Chandra, S., Lata, H., ElSohly, M. A., Walker, L. A., & Potter, D. (2017). Cannabis cultivation: Methodological issues for obtaining medical-grade product. *Epilepsy & Behavior*, 70, 302–312.
- Chandra, S., Bandopadhyay, R., Kumar, V., & Chandra, R. (2010). Acclimatization of tissue cultured plantlets: From laboratory to land. *Biotechnology Letters*, 32(9), 1199–1203.
- Chaohua, C., Gonggu, Z., Lining, Z., Chunsheng, G., Qing, T., Jianhua, C., Xinbo, G., Dingxiang, P., & Jianguang, S. (2016). A rapid shoot regeneration protocol from the cotyledons of hemp (*Cannabis sativa* L.). *Industrial Crops and Products*, 83, 61–65.

- Clarke, R. C., & Merlin, M. D. (2016). Cannabis domestication, breeding history, present-day genetic diversity, and future prospects. *Critical Reviews in Plant Sciences*, 35(5-6), 293-327.
- Crini, G., Lichtfouse, E., Chanet, G., & Morin-Crini, N. (2020). Traditional and new applications of hemp. In G. Crini & E. Lichtfouse (Eds.), *Sustainable agriculture reviews* (pp. 37-87). Springer.
- Dev, R., Singh, S., Dayal, V., Kumar, K., & Singh, T. (2019). Standardization of in vitro hardening strategies for tissue cultured wine grape (*Vitis vinifera* L.) genotypes. *International Journal of Current Microbiology and Applied Sciences*, 8(2), 2108-2119.
- Devi, P. S., Arundathi, A., & Rao, T. R. (2011). Multiple shoot induction and regeneration of whole plants from cotyledonary node and nodal explants of *Sterculia urens* Roxb. a gum yielding tree. *Journal of Plant Biochemistry and Biotechnology*, 20, 161-165.
- Dimitrova, N., & Nacheva, L. (2021). An optimized micropropagation protocol by ex vitro rooting of pear rootstock OHF 333 (*Pyrus communis* L.). *Acta Agrobotanica*, 74, 1.
- Dinani, E. T., Shukla, M. R., Turi, C. E., Sullivan, J. A., & Saxena, P. K. (2018). Thidiazuron: Modulator of morphogenesis in vitro. In *Thidiazuron: From urea derivative to plant growth regulator* (pp. 1-36). Springer.
- Dushimimana, C., Sakha, M. A., Korir, M. J., Jefwa, J., Vandenabeele, J., Magomere, T., Mutitu, E., Mulatya, J., Olubayo, F., Smagghe, G., & Werbrout, S. (2022). Early growth performance of in vitro raised *Melia volkensii* Gürke plantlets in response to beneficial microorganisms under semi-arid conditions. *Plants*, 11(10), 1300.
- Gehl, C., Li, G., & Serek, M. (2020). An efficient protocol for *Agrobacterium*-mediated transformation and regeneration of *Campanula medium* (Canterbury bells) based on leaf disc explants. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 140, 635-645.
- González, M. M., Yañuk, G., Sannazzaro, A., Butassi, E., Di Liberto, M., Perez, E., ... & Clemente, M. (2025). Optimization of a *Cannabis sativa* micropropagation protocol (chemotype III) to preserve its cannabinoid profile. *bioRxiv*, 2025-05.
- Grzelak, M., Pacholczak, A., & Nowakowska, K. (2024). Challenges and insights in the acclimatization step of micropropagated woody plants. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 159(3).
- Holmes, J. E., Lung, S., Collyer, D., & Punja, Z. K. (2021). Variables affecting shoot growth and plantlet recovery in tissue cultures of drug-type *Cannabis sativa* L. *Frontiers in Plant Science*, 12, 732344.
- Huetteman, C. A., & Preece, J. E. (1993). Thidiazuron: A potent cytokinin for woody plant tissue culture. *Plant Cell, Tissue and Organ Culture*, 33, 105-119.
- Hurgobin, B., Tamiru-Oli, M., Welling, M. T., Doblin, M. S., Bacic, A., Whelan, J., & Lewsey, M. G. (2021). Recent advances in *Cannabis sativa* genomics research. *New Phytologist*, 230(1), 73-89.
- Ingvarsdén, C. R., & Brinch-Pedersen, H. (2023). Challenges and potentials of new breeding techniques in *Cannabis sativa*. *Frontiers in Plant Science*, 14, 1154332.
- Ioannidis, K., Dadiotis, E., Mitsis, V., Melliou, E., & Magiatis, P. (2020). Biotechnological approaches on two high CBD and CBG *Cannabis sativa* L. (Cannabaceae) varieties: In vitro regeneration and phytochemical consistency evaluation of micropropagated plants using quantitative ¹H-NMR. *Molecules*, 25(24), 5928.
- Ioannidis, K., Tomprou, I., Mitsis, V., & Koropoulis, P. (2022). Genetic evaluation of in vitro micropropagated and regenerated plants of *Cannabis sativa* L. using SSR molecular markers. *Plants*, 11(19), 2569.
- Isah, T. (2020). Nodal segment explant type and preconditioning influence in vitro shoot morphogenesis in *Ginkgo biloba* L. *Plant Physiology Reports*, 25(1), 74-86.
- Kose, M. S. H., Dogan, M., & Sadi, G. (2021). Enhanced in vitro shoot proliferation through nodal explants of *Staurogyne repens* (Nees) Kuntze. *Biologia*, 76(3), 1053-1061.
- Lata, H., Chandra, S., Khan, I., & ElSohly, M. A. (2009). Thidiazuron-induced high-frequency direct shoot organogenesis of *Cannabis sativa* L. *In Vitro Cellular & Developmental Biology - Plant*, 45, 12-19.
- Lata, H., Chandra, S., Techen, N., Khan, I., & ElSohly, M. A. (2010). Assessment of the genetic stability of micropropagated plants of *Cannabis sativa* by ISSR markers. *Planta Medica*, 76, 97-100.
- Lata, H., Chandra, S., Techen, N., Khan, I., & ElSohly, M. A. (2016). In vitro mass propagation of *Cannabis sativa* L.: A protocol refinement using novel aromatic cytokinin meta-topolin and the

- assessment of ecophysiological, biochemical and genetic fidelity of micropropagated plants. *Journal of Applied Research on Medicinal and Aromatic Plants*, 3, 18–26.
- Lata, H., Chandra, S., Khan, I. A., & ElSohly, M. A. (2017). Micropropagation of *Cannabis sativa* L.—an update. In *Cannabis sativa L.-botany and biotechnology* (pp. 285-297). Cham: Springer International Publishing.
- Li, Y., Hu, J., Xiao, J., Guo, G., & Jeong, B. R. (2021). Foliar thidiazuron promotes the growth of axillary buds in strawberry. *Agronomy*, 11(3), 594.
- Liu, Y., Liu, H. Y., Li, S. H., Ma, W., Wu, D. T., Li, H. B., & Gan, R. Y. (2022). *Cannabis sativa* bioactive compounds and their extraction, separation, purification, and identification technologies: An updated review. *TrAC Trends in Analytical Chemistry*, 149, 116554.
- Mahendra, R., Chauhan, N., Sharma, J. B., Rana, K., & Bakshi, M. (2020). Ex-vitro establishment of tissue cultured plants in fruit crops - A review. *International Journal of Current Microbiology and Applied Sciences*, 9(11), 3321–3329.
- Marks, T. R., & Myers, P. E. (1992). Effect of explant location upon early culture development in vitro. *Journal of Horticultural Science*, 67(5), 583–591.
- Martini, A. N., Vlachou, G., & Papafiotiou, M. (2022). Effect of explant origin and medium plant growth regulators on in vitro shoot proliferation and rooting of *Salvia tomentosa*, a native sage of the Northeastern Mediterranean Basin. *Agronomy*, 12(8), 1889.
- Monthony, A. S., Kyne, S. T., Grainger, C. M., & Jones, A. M. P. (2021). Recalcitrance of *Cannabis sativa* to de novo regeneration: A multi-genotype replication study. *PLOS ONE*, 16(8), e0235525.
- Movahedi, M., Ghasemi-Omran, V., & Torabi, S. (2015). The effect of different concentrations of TDZ and BA on in vitro regeneration of Iranian cannabis (*Cannabis sativa*) using cotyledon and epicotyl explants. *Journal of Plant Molecular Breeding*, 3(2), 20–27.
- Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, 15, 473–497.
- Murphy, R., & Adelberg, J. (2021). Physical factors increased quantity and quality of micropropagated shoots of *Cannabis sativa* L. in a repeated harvest system with ex vitro rooting. *In Vitro Cellular & Developmental Biology - Plant*, 1–9.
- Page, S. R. G., Monthony, A. S., & Jones, A. M. P. (2020). Basal media optimization for the micropropagation and callogenesis of *Cannabis sativa* L. *bioRxiv*, 1–23.
- Page, S. R. G., Monthony, A. S., & Jones, A. M. P. (2021). DKW basal salts improve micropropagation and callogenesis compared with MS basal salts in multiple commercial cultivars of *Cannabis sativa*. *Botany*, 99(5), 269–279.
- Ranaweera, K. K., Gunasekara, M. T. K., & Eeswara, J. P. (2013). Ex vitro rooting: A low cost micropropagation technique for tea (*Camellia sinensis* (L.) O. Kuntz) hybrids. *Scientia Horticulturae*, 155, 8–14.
- Rao, A. N., & Lee, S. K. (1986). Overview of the in vitro propagation of woody plants and plantation crops. In L. A. Withers & P. G. Aldeson (Eds.), *Plant tissue culture and its agricultural application* (pp. 123–138). Cambridge University Press.
- Razumova, O. V., Alexandrov, O. S., Divashuk, M. G., Sukhorada, T. I., & Karlov, G. I. (2016). Molecular cytogenetic analysis of monoecious hemp (*Cannabis sativa* L.) cultivars reveals its karyotype variations and sex chromosomes constitution. *Protoplasma*, 253, 895–901.
- Shekhawat, M. S., Manokari, M., & Ravindran, C. P. (2015). An improved micropropagation protocol by ex vitro rooting of *Passiflora edulis* Sims. f. *flavicarpa* Deg. through nodal segment culture. *Scientifica*, 2015(1), 578676.
- Smýkalová, I., Vrbová, M., Cvečková, M., Plačková, L., Žukauskaitė, A., Zatloukal, M., & Griga, M. (2019). The effects of novel synthetic cytokinin derivatives and endogenous cytokinins on the in vitro growth responses of hemp (*Cannabis sativa* L.) explants. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 139, 381–394.
- Stephen, C., Zayas, V. A., Galic, A., & Bridgen, M. P. (2023). Micropropagation of hemp (*Cannabis sativa* L.). *HortScience*, 58(3), 307–316.
- Talei, D., Shams, A., & Khayam Nekouei, M. (2025). A comprehensive review of cannabis as a crucial pharmaceutical plant and its efficient propagation methods. *ACS Agricultural Science & Technology*, 5(7), 1191–1214.

- Thiem, B., Hermosaningtyas, A. A., Budzianowska, A., & Kikowska, M. (2023). Polish contributions in developing medicinal plant in vitro propagation system. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 155(1), 1–17.
- Wang, F., Li, Y., Pang, Y., Hu, J., Kang, X., & Qian, C. (2025). Thidiazuron enhances strawberry shoot multiplication by regulating hormone signal transduction pathways. *International Journal of Molecular Sciences*, 26(9), 4060.
- Wang, R., He, L. S., Xia, B., Tong, J. F., Li, N., & Peng, F. (2009). A micropropagation system for cloning of hemp (*Cannabis sativa* L.) by shoot tip culture. *Pakistan Journal of Botany*, 41(2), 603–608.
- Wongchaya, P., Bansra, T., & Mahadtanapuk, S. (2025). Rapid micro-propagation of disease-free hemp (*Cannabis sativa* L.) to improve industrial production. *Trends in Sciences*, 22(1), 9057.
- Wróbel, T., Dreger, M., Wielgu, K., & Słomski, R. (2022). Modified nodal cuttings and shoot tips protocol for rapid regeneration of *Cannabis sativa* L. *Journal of Natural Fibers*, 19(2), 536–545.
- Xiong, Y., Chen, S., Wei, Z., Chen, X., Guo, B., Zhang, T., ... & Zeng, S. (2022). Transcriptomic analyses provide insight into adventitious root formation of *Euryodendron excelsum* HT Chang during ex vitro rooting. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 148(3), 649–666.
- Zhang, Z., Sun, Y., & Li, Y. (2020). Plant rejuvenation: From phenotypes to mechanisms. *Plant Cell Reports*, 39, 1249–1262.