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Breaking seed dormancy and regeneration in *Cannabis sativa* L.

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Abstract

Cannabis sativa L. is an important medicinal plant species that grow under natural conditions and has been legalized in 20 out of 81 Turkish provinces. The female inflorescence is a highly branched compound raceme with indeterminate habit of growth. This results in different maturing of seeds on the inflorescence and induce physiological dormancy on seeds. The study aimed to improve seed germination percentage using various concentrations of GA_3, GA_3 + BAP, germination on water and water solidified with agar, MS or Gamborg B5 medium. The results showed that the best seed germination was noted on Gamborg B5 medium. Different explants were used to regenerated plantlets on Gamborg B5 medium. All explants were suitable for callus regeneration variably. Only the stem nodes of Samsun Vezirköprü were suitable to induce shoots and plantlets. These plantlets were acclimatized on clay loam soils and transferred to field condition during October 2020, where they acclimatized successfully. These studies provide an effective insight into the mechanism seed dormancy in *C. sativa.* Further studies using other plant growth regulator concentrations will improve shoot regeneration and aid in utilizing the methods for breeding purpose.

Keywords: Hemp, *In vitro*, *Mass* propagation, Seed germination, TDZ

Introduction

Cannabis sativa L. (family *Cannabaceae*) is an importnt plant species that has been cultivated in many Asian and European countries as annual herbaceous, multi-purpose plant species used in medicinal or palliative care systems since 2700's years before the Common Era (BCE) (Schäfer, 2005, Schumacher, et al., 2020). Cannabidiol and Cannabidiolic acid are the important and abundant phytocannabinoids in *C. sativa* cultivars in general, but some of them biosynthesize cannabigerol as the major constituent compounds (Hanuš, et al., 2016) that are evaluated as non-psychotropic compounds with potential therapeutic uses. They are considered neuroprotective, anti-rheumatoid anxiolytic, anti-nausea, anti-spasmodic and used for the treatment of arthritis (Bonini, et al., 2018, Hanuš, et al., 2016,) and cancer (Sánchez et al., 2001, Blázquez et al., 2004, Śledziński, et al., 2018), appetite loss and prevent vomiting (Abrams, 2016).

The stem is herbaceous in the first development period, with high sap, and takes a corrugated arthritis body appearance in the later stages of growth. The plants contain 70% cellulose (with ~22% hemicellulose and ~45% carbon). Its fiber is used to make a durable and cost-effective thread (Seher et al., 2020). It is also a rich source of raw material in paper, medicinal or pharmacological products (e.g., phytocannabinoids, terpenes, and phenolic compounds) (Bonini, et al., 2018, Hanuš, et al., 2016).

It is allelopathic and could be used in soil phytoremediation, with the ability to prevent or suppress both weeds and soil pathogens (Adesina, et al., 2020).

Its cultivation in many countries of the world including Turkey remained banned for many years in accordance with global trends because of psychoactive contents. However, its cultivation has been allowed in 20 out of 81 provinces according to the "Regulation on *Cannabis* Growing and Control." (Official Gazette, 2016, 2021). *C. sativa* plants are produced industrially in 36 countries in the world (Aydogan, 2020). The global market US $ 4 billion in 2017, US $ 4.7 billion in 2018, for *C. sativa* is expected to reach US $ 11 billion in 2025 (FAO, 2019).

When the data of TUİK for 1998-2018 is reviewed, there is a decline in *C. sativa* production,

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which reached to total production of 160 tons in 2001(TSI, 2020).

C. sativa is a dichotomous plant with male and female floral organs on different plants with an indeterminate type of growth habit. The most important step in production of *Cannabis* is understanding mechanism of seed germination, plant survival and its harvest of seeds, alkaloids and fibre from the plants at economic level. The Inflorescence is highly branched with a compound raceme and pistillate flowers (Spitzer-Rimon, et al. 2019, Bernstein et al. 2019). The development of the inflorescence is acropetal and lateral racemes are produced prior to terminal flower differentiation (Spitzer-Rimon, et al. 2019, Hall et al., 2012).

The most important step in production of *Cannabis* is understanding mechanism of floral and pollination biology along with seed germination (Spitzer-Rimon, et al. 2019, Strzelczyk et al. 2021). The *Cannabis* or hemp seed germination testing protocols are mentioned in ISTA rules (ISTA 2021), Quality plants and seed production is very difficult in *Cannbis* due to unavailability of registered cultivars and varieities world over in general.

Therefore, seed germination percentage and germination performance depends on the environment and varies from plant to plant. This ends up in several types of dormancies that could be released using various phytohormones or chemical treatments (Ewel, et al., 2019, Jovičić, et al. 2019, Green, et al., 2016, Geneve, 2016) that could end up in variable results. Therefore, there is need to establish simplest methods for successful germination and cultivation (Sera, et al.2017).

Chemical pre germination treatments help to stimulate and increase seed germination and quality (Walck, et al., 2005). Therefore, it is necessary to evaluate chemical treatments to improve its seed germination to enhance production and yields (Chahtane, et al., 2017). Hence, there is a need to design experiments for increased seed germination considering the ever-increasing need for plant biomass and pharmacological products, the World over.

The current study was conducted to break seed dormancy and evaluate the effect of different osmo and hydro priming treatments to successful germination of Samsun Vezirköprü and Uşak populations under *in vitro* conditions.

Materıals and Methods

Seed Material

Seeds belonging to the Uşak and Samsun Vezirköprü populations used in the study were obtained from the Department of Field Crops, Faculty of Agriculture, Uşak University, Turkey.

Methods

Sterilization of Equipment

All laboratory equipment made of glass used in the study were sterilized by keeping them in the oven at 160 ° C for 2 hours. The rest of the material including culture boxes and culture media used in the study were sterilized using autoclave under 4.5 kPa atmospheric pressure and 121 °C for 20 minutes. The forceps and scalpels were cleaned with 70% (v / h) alcohol and then sterilized at 250 °C with a steril 250 sterilizer device in a laminar airflow cabinet.

Surface Sterilization of Seeds

The seeds were shaken in a laminar flow cabinet under sterile conditions on a magnetic stirrer for 15 minutes by dipping and shaking them in 50, 70, and 90% commercial bleach (ACE - Turkey containing 5% sodium hypochlorite-NaOCl) at room temperature. Thereafter, the seeds were rinsed for 3 × 3 min with sterile distilled water at room temperature.

Contamination in the culture medium or over explants was monitored for one week after planting the seeds in the culture medium. The serilized mature seeds were rinsed 3 × 3 min and used as control treatment. The experiments were repeated 3 times.

Germination of Seeds and Regeneration of *C. sativa* Seeds

Sterilized *C. sativa* seeds were cultured in 3 replications with 5 seeds in each Petri dish, and they were kept in the growth cabinet for 10 days to form seedlings. These experiments were also repeated 3 times.

The optimal concentration of bleach was determined after the sterilization and was used in the rest of the studies.

Following treatments were given to break seed dormancy and seed germination

Seed Dormancy Break

i. Treatment with MS medium containing 0.2, 0.4, 0.6, 0.8, 1, 1.2 mg/l gibberellic acid (GA_3) (6 treatments)

ii. Treatment with MS medium containing 0.4 mg/l GA_3 + 0.5, 0.8, 1 mg/l BAP (4 treatments)

iii. Treatment with water and solidified with agar.

iv. Treatment with MS or Gamborg B5 medium.

Regeneration

Upper portion of the leaf, central portion of the leaf, the lower portion of the leaf, petiole, Stem node, and internode explants of 10 days-old *C. sativa* plantlets were treated with ½ ×Gamborg B5 containing 0.1, 0.2, 0.3, 0.4, 0.5 mg/l TDZ solidified with 3.5, 5 g/l agar supplemented with 10 g/l sucrose.

The pH of the nutrient medium was adjusted to 5.7 ± 0.1 using 1 N NaOH or 1 N HCl. Subsequently, sterilization was provided by keeping the respective culture medium under 4.5 kPa pressure and at 121° C for 20 minutes.

The seeds were cultured in a dark in a growth chamber for 12 days at 25 ± 1 °C. Thereafter, the explants were taken from these plantlets as described above.

The cultures were transferred to a chamber with 16 hours light and 8 hours dark photoperiod at 24°C temperature.

Acclimatisation

The growing plants were acclimatised in seedling trays covered with two vented covers. The plantlets continued to grow in these vented trays until they showed the sighns of growth (15 days). Thereafter, the plants were transferred to one litre plastic pots filled with peat moss and transferred to the greenhouse.

Evaluation of Data for Statistical Analysis

The experimental pattern consisted of 3 replications for each treatment using 100 × 10 mm Petri dishes. IBM SPSS 26 computer program was used for the analysis of variance. The results of each experiment were compared with One Way ANOVA. LSD or Duncan test was performed to separate statistically different means in each experiment unless otherwise mentioned. The arcsin transformation was applied to the percent values before statistical analysis (Snedecor, & Cochran, 1967).

Results and Discussion

Effect of Sterilization Treatments on *C. Sativa* Seeds

Effect of Sterilization treatments on *C. Sativa* seeds

Sodium hypochlorite often purchased as bleach, is the most commonly used chemical for the surface sterilization of seeds. Commercial bleach is 5-5.25% sodium hypochlorite. Seed material is often immersed/mixed in this solution singly or with a magnetic stirrer for 10 - 20 minutes or more.

Optimization studies are carried out to experimentally determine a balance between concentration and time due to phytotoxicity for each explant type. The results showed that all concentrations of commercial bleach were appropriate for seed sterilization. However, the percentage of seed germination varied using 50, 70, and 90% concentration of commercial bleach showing a range of 33.333-42.222% and 35.556-53.33 for population Uşak and Samsun Vezirköprü respectively (Figure 1). The maximum germination percentage was determined as 53.33%. Similarly, the use of bleach has been found appropriate in many previous sterilization studies of other plants (Kai, et al., 2007, Daud et al., 2012, Hesami, et al., 2019, Ines, et al., 2013, Bello, et al., 2018).

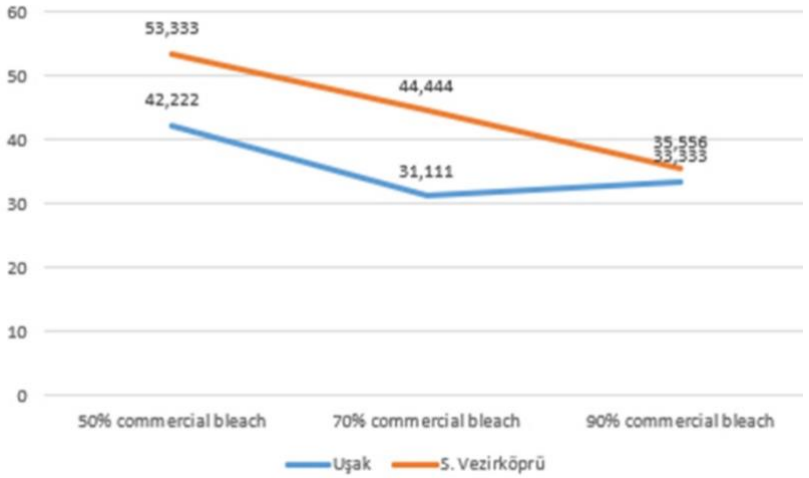

Figure 1. Effect of different concentrations of commercial bleach on *C. sativa* seeds germination percentage

Seed Germination

Effect of GA_3 Doses on Germination

Apart from these possible causes of dormancy, which directly or indirectly affect seed metabolism of carbohydrates, proteins, and other reserves in the germination process, dormancy can also be attributed to a balance between growth-regulating hormones that play a fundamental role in the seed germination process. The results indicated a germination percentage of 13.333-40.000% in population Uşak and 20.000-53.333% in population Samsun Vezirköprü. The highest germination in both populations was noted after treatment with 0.4 mg/l GA_3 (Figure 2).

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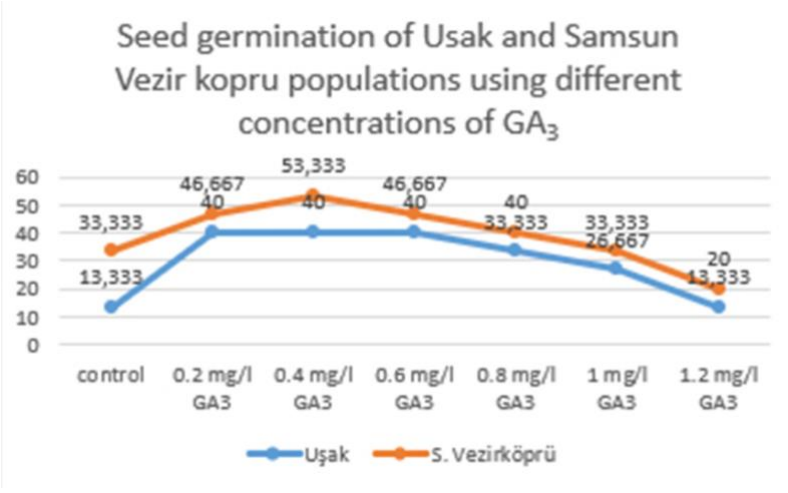

Figure 2. *C. sativa* seeds germinating using different concentrations of GA_3

Gibberellic acid plays a role in overcoming dormancy as well as in controlling the hydrolysis of reserves. The presence of sufficient levels of this acid in seeds stimulates the synthesis, activation, and secretion of hydrolytic enzymes, especially α-amylase, releasing reducing sugars and amino acids necessary for embryo growth (Khan, 1971). External application of Gibberellic acid (GA_3) is one of the hormones suggested to control and break seed dormancy and induce germination (Ritchie, & Gilroy, 1998, Greipsson, 2001).

Effect of GA_3 and different BAP Concentrations on Seed Germination;

The previous experiment showed the highest germination percentage of *C. sativa* seeds of two populations using 0.40 mg/l GA_3. Using this concentration as control, this study compared the effects of 0.40 mg/l GA_3 Control treatment, 0.50 mg/l BAP+0.40 mg/l BAP, 0.40 mg/l GA_3 + 0.80, 1mg / L BAP (Figure 3).

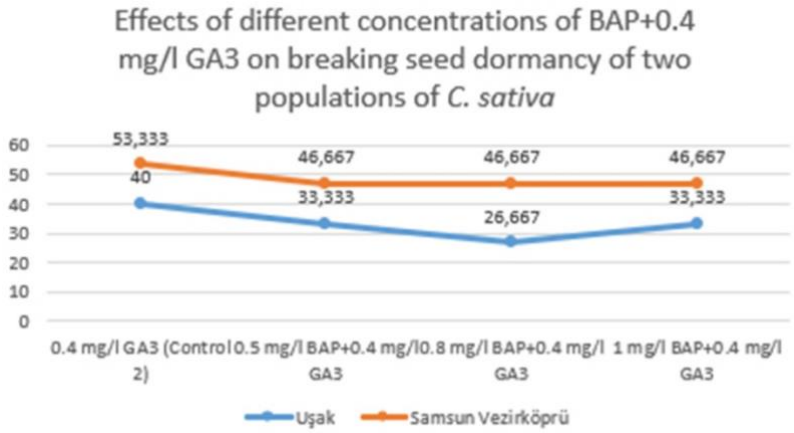

Figure 3. Determination of the effect of different concentrations of BAP+GA_3 on seed germination of *C. Sativa*

Plant hormones have been found to play an important role in the germination process (Nadjafia, et al., 2006). The experimental results showed that BAP+GA_3 combinations had significant inhibitory effects on seed germination percentage and were not as effective, as when GA_3 was used singly. The germination rate varied between 20-33.333% on the Uşak population. The seed germination percentage of the Samsun Vezirköprü population ranged between 26.667-46.667%. The highest seed germination percentage did not show an improvement over control treatment using 0.40 mg/l GA_3 singly.

Effect of Pure Sterile Water on Germination;

Hydro-priming and osmopriming seed pretreatment techniques have been applied to enhance the germination of *C. sativa* populations (Ashraf, et al., 2005, Paparella, et al., 2015).

This experiment compared the effects of water and water solidified with agar on seed germination. It was observed that seed germination percentage using water singly was superior compared to the using water solidified with agar. It showed germination of 50% in Uşak and 63.33% in S. Vezirköprü population (Figure 4). Agar was inhibitory ending up with a germination percentage of 24.67% in the Uşak and 26.67% in the

VezirköprüSamsun Vezirköprü population. The results of this study show that hydro-priming application is effective in overcoming dormancy in *C. sativa* seeds compared to osmopriming using water solidified with agar. It was found that *C. sativa* seeds have physical dormancy due to hard seed coats.

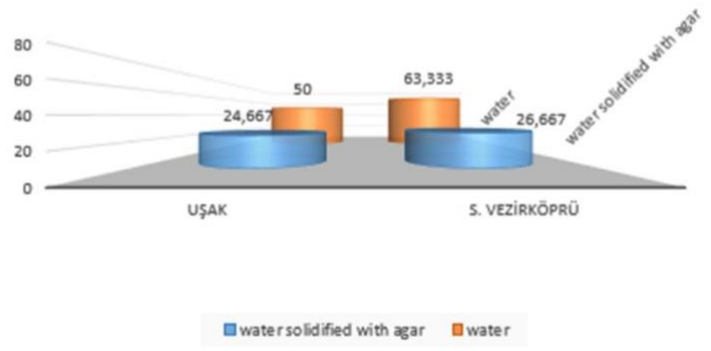

Figure 4. Determination of the effect of sterile pure water on germination in *C. Sativa* plants

Effects MS and Gamborg B5 Medium on Seed Germination;

MS medium is the most widely used medium for plant tissue cultures. It was developed for tobacco tissue culture by Murashige and Skoog (MS) (Sattar, et al., 2010, Owen, et al., 1991, Sarwar, et al., 2009, Khan, et al., 1999). The key feature of MS medium is the very high concentration of nitrate, potassium, and ammonia. Glycine, one of the vitamins, is present in MS medium, which is not present in Gamborg B5 medium (Gamborg, et al., 1976). Inorganic nutrient levels in Gamborg B5 medium are lower compared to the MS medium. Nicotinic Acid, Pyridoxine HCl, and Thiamine HCl are present at a higher percentage compared to MS medium. Inhibition was detected in MS medium containing high nitrogen and K and small amounts of vitamins compared to Gamborg B5 medium.

C. sativa showed seed germination of 26,666% and 33,333% using Uşak and Samsun Vezirköprü population on MS medium (Figure 5). However, an improved seed germination percentage of 66.666% in Uşak and 73.33% in S. Vezirköprü population was noted using Gamborg B5 medium. The results of this study showed that Gamborg B5 medium was more effective in seed germination compared to the MS medium. Thereafter, all seeds were germinated using this methodology.

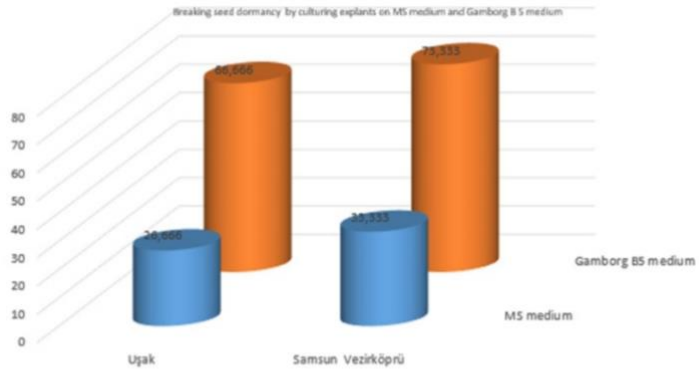

Figure 5. A comparison between Germination percentage on MS and Gamborg B5 medium

Micropropagation Studies

TDZ concentrations have been found to be beneficial in shoot proliferation in a number of explants taken from several plant species. TDZ can inhibit shoot elongation and regeneration (Mok, & Mok, 1985, Gribaudo, & Fronda, 1991, Mok, et al., 1982, Khawar, 2004). Callus formation was noted on all explants but they did not induce any

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adventitious shoots except on calli induced on stem nodes. Shoot induction was noted on stem node explants only (Figure 6). Either callus formation or shoot induction was noted on the Uşak population. Callus induction was noted on the upper portion of the leaf, central portion of the leaf, lower portion of leaf, petiole, internode, and stem nodes explants of Samsun Vezirköprü population showing the percentage of 33.333-66.67%, 33.333-50%, 25-66.667%, 0.667-21.667%, 66.67-83.333%, 25.00-66.667% respectively (Table 1).

Minimum and maximum callus formation was noted on 0.1 and 0.5 mg/l TDZ with the exception of petiole and stem node explant; where maximum callus formation was noted on 0.4 mg/l TDZ. Each increase in the concentration of TDZ from 0.1 mg/l to 0.3 mg/l showed promotory effect on callus induction from stem node explants with shoot regeneration of 75.335% and 4.593 shoots per explant (Figure 6 a,b,c,d,e, f, g, h) Samsun Vezirköprü population.

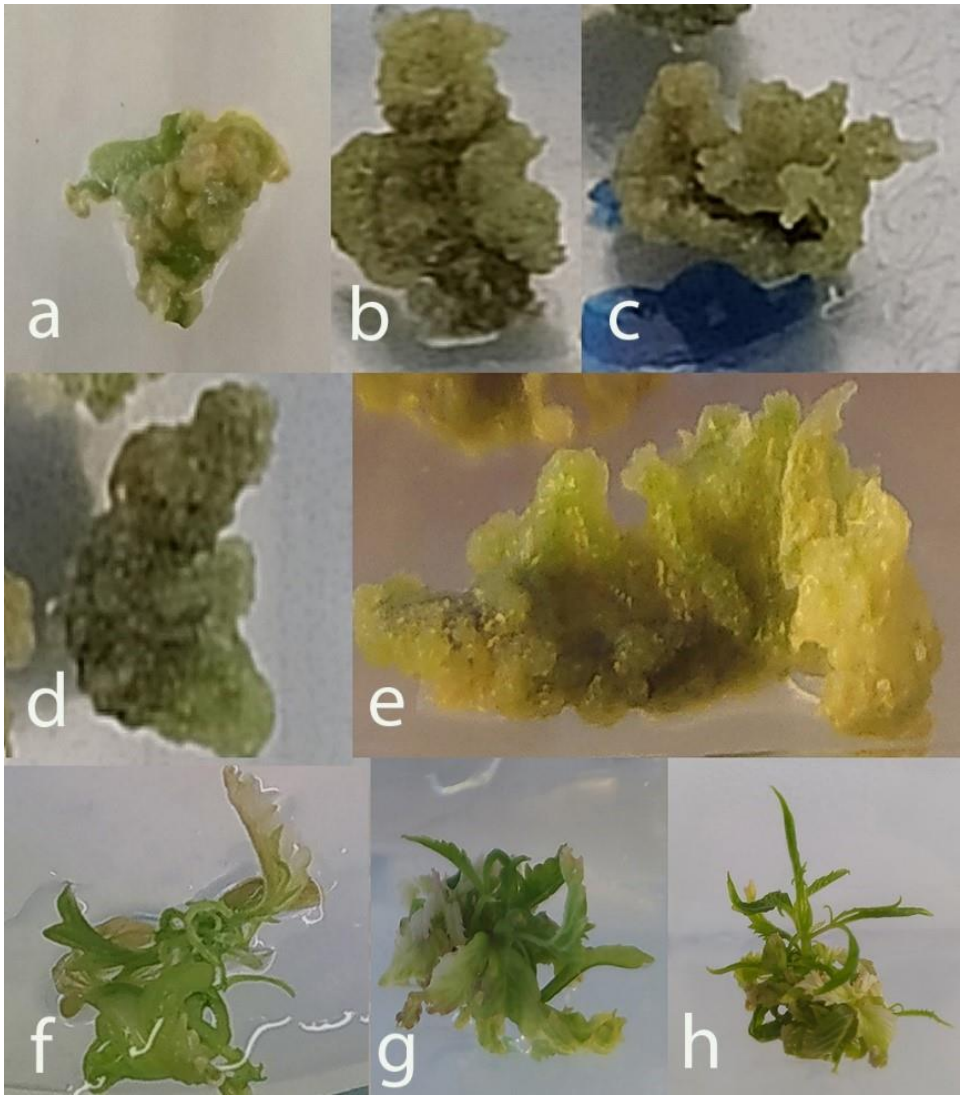

Figure 6. Observing the effect of TDZ doses on different explants (a), upper portion of leaf, (b), central portion of leaf, (c), lower portion of leaf, (d), petiol, (e), stem node (f) ınternode explants Fig a, b, c, d, f, g, h

Table 1. Duncan test results to determine the effects of varying TDZ doses on different explants of *C. Sativa* plants

Explant type	0.1 mg/l TDZ			0.2 mg/l TDZ		
	Swelling percentage (%)	Shoot regeneration percentage (%)	Number of shoots per explant	Swelling/precallusing percentage (%)	Shoot regeneration percentage (%)	Number of shoots per explant
Upper portion of leaf	33,333b*	0.000	0.000	41,667ab	0.000	0.000
Central portion of leaf	33,333b	0.000	0.000	41,667ab	0.000	0.000
Lower portion of leaf,	25,000b	0.000	0.000	50,000ab	0.000	0.000
Petiol	0,667b	0.000	0.000	8,333b	0.000	0.000
Internode explants	25,000b	0.000	0.000	41,667b	0.000	0.000
Stem node	66,667b	0.000	0.000	58,333b	0.000	0.000
	0.3 mg/l TDZ			**0.4 mg/l TDZ**		
Explant type	**Swelling/precallusing percentage (%)**	**Shoot regeneration percentage (%)**	**Number of shoots per explant**	**Swelling/precallusing percentage (%)**	**Shoot regeneration percentage (%)**	**Number of shoots per explant**
Upper portion of leaf	66,667a	0.000	0.000	58,333ab	0.000	0.000
Central portion of leaf	66,667a	0.000	0.000	50,000ab	0.000	0.000
Lower portion of leaf,	50,000ab	0.000	0.000	41,667ab	0.000	0.000
Petiol	66,667a	0.000	0.000	21,667a	0.000	0.000
Internode explants	66,667b	0.000	0.000	25,000b	0.000	0.000
Stem node	16,667a	0.000	0.000	83,333a	75.334	0.000
Explant type	**0.5 mg/l TDZ**					
	Swelling percentage (%)	**Shoot regeneration percentage (%)**	**Number of shoots per explant**			
Upper portion of leaf	33,333b	0.000	0.000			
Central portion of leaf	33,333b	0.000	0.000			
Lower portion of leaf,	33,333b	0.000	0.000			
Petiol	8,333b	0.000	0.000			
Internode explants	25,000b	0.000	0.000			
Stem node	58,333b	75.335	4.593			

*****All nean values given in a column are significantly different using Duncan's Multiple Range Test at 0.05 level of signifcance

Rooting and Acclimatisation

Adaptation of plants obtained from laboratory studies to external conditions is important for ensuring sustainability. All developing shoots on stem node explants rooted in the regeneration medium. Therefore no separate medium containing auxins were used for rooting. These plants were transferred to transparent plastic pots for acclimatization in the growth room (Figure 7).

The *C. sativa* has high adaptability and has spread from the subtropical zone to the temperate zone climate line. *C. sativa* grows naturally or is grown on a limited scale. The amount of moisture in the soil is important before planting. The plant has a high water requirement and the rainfall requirement is high. Since the *C. sativa* plant is very sensitive to temperatures below 0°C, it is badly damaged at temperatures lower than -5 ° C, it needs at least 150 days of maturity not lower than 0°C degrees for seed production and 120 days for quality fiber production (Merve and Orhan, 2020).

The most suitable soils for *C. sativa* plants are medium-heavy, well-drained, airy, deep, fertile in nutrients, soil pH between 6-7.5, loose, loamy and rich in organic matter, calcareous, alluvial soils. Sandy soils, slightly acidic, slightly arid, loamy, and heavy soils, and soils with low permeability and poor drainage are not suitable for *C. sativa* cultivation (Merve and Orhan, 2020). However, when the climatic conditions are evaluated (Figure 8), the *C. sativa* plant, which has a very low resistance to temperatures below 0°C, is completely

damaged at temperatures lower than -5 ° C (Merve, & Orhan, 2020). Therefore it was determined that the plants that acclimatized well during October could not withstand the colds of December when the temperature drops below 5°C and it was damaged.

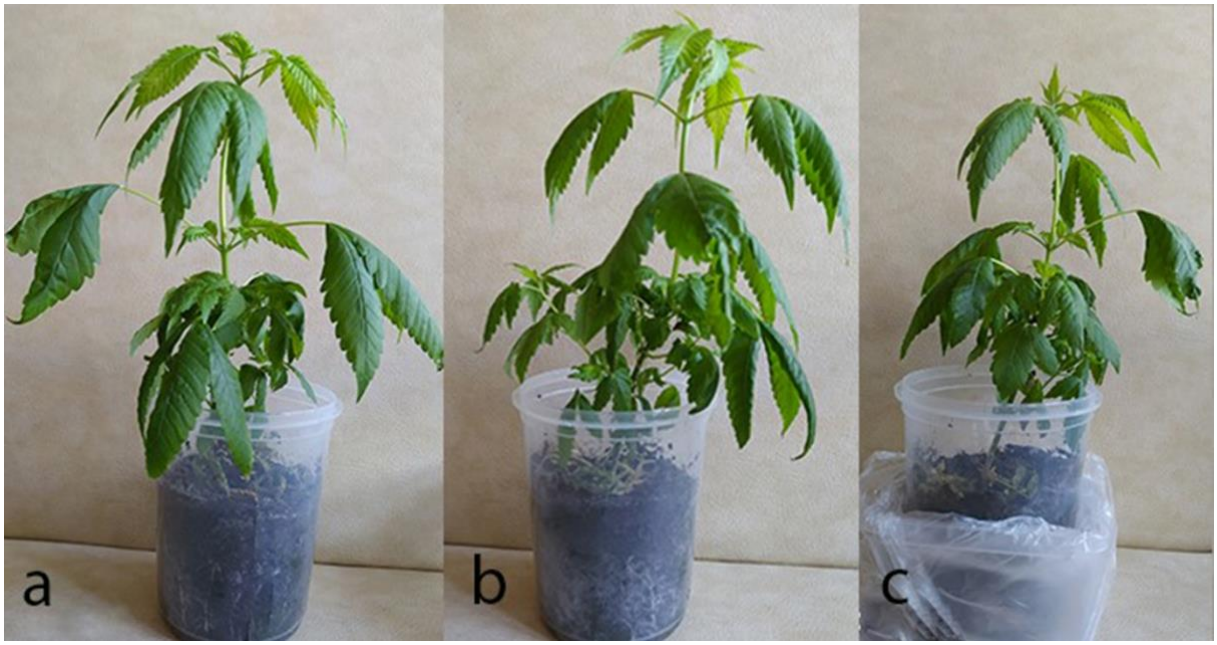

Figure 7. The acclimatization of the plants of *In vitro* cultured plantlets of (a, b, c) Samsun Vezirköprü population to external conditions in the growth cabinet before transfer to external conditions

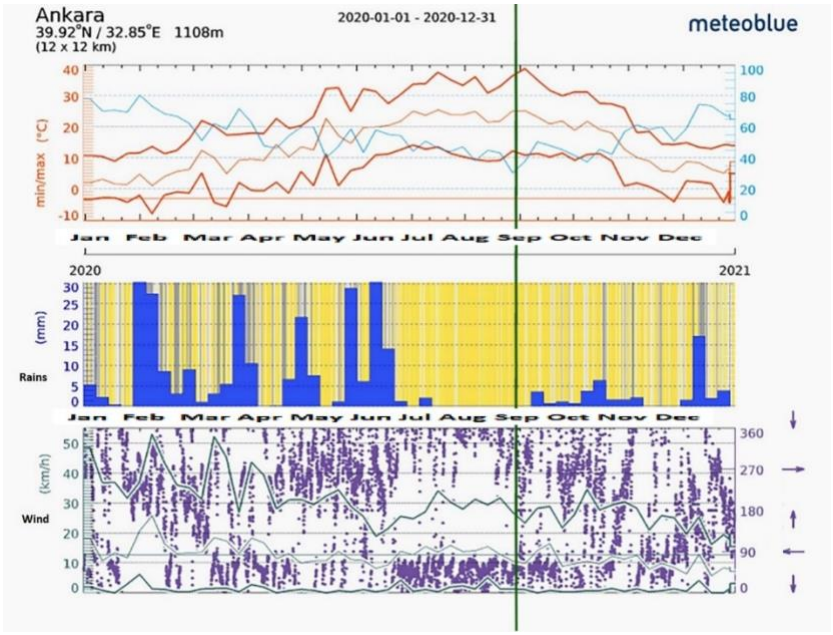

Figure 8. Temperature, precipitation and wind data of the trial site for 2020 (https://www.meteoblue.com/tr/hava/historyclimate/weatherarchive/ankara_t%c3%bcrkiye_323786?fcstlength=1y&year=2020&month=2)

Conclusion

The Uşak and Samsun Vezirköprü populations of *C. sativa* were used in the study. The results of this study optimized conditions for seed germination of two *C. sativa* populations. Samsun Vezirköprü population was found vigorous compared to the Uşak population. The results indicated that the *C. sativa* is suitable for spring sowing under hot humid continental climate of Ankara. It was indicated that further studies should be addressed to the phytochemical behavior of these populations. These studies will be of significant importance for further studies related to the breeding of new cultivars from these populations.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

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Data availability

Not applicable.

Consent for publication

Not applicable.

References

Abrams, D. I. (2016). Integrating *cannabis* into clinical cancer care. *Current Oncology*, *23*(Suppl 2), S8. DOI: https://doi.org/10.3747/co.23.3099

Adesina, I., Bhowmik, A., Sharma, H., & Shahbazi, A. (2020). A review on the current state of knowledge of growing conditions, agronomic soil health practices and utilities of hemp in the United States. *Agriculture*, *10*(4), 129. DOI: https://doi.org/10.3390/agriculture10040129

Ashraf, M.; Foolad, M.R. (2005). Pre-sowing seed treatment –A shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. *Advances in Agronomy*, *2005*, 88, 223–271. DOI: 10.1016/S0065-2113(05)88006-X

Aydogan, M., Terzi, Y. E., Gizlenci, S., Mustafa, A. C. A. R., Alpay, E. S. E. N., & Meral, H. (2020). Turkiye'de kenevir yetiştiriciliginin ekonomik olarak yapılabilirligi: Samsun ili Vezirköprü ilcesi ornegi. *Anadolu Tarım Bilimleri Dergisi*, *35*(1), 35-50. DOI: https://doi.org/10.7161/omuanajas.602585

Bello, O. A., Esan, E. B., & Obembe, O. O. (2018). Establishing surface sterilization protocol for nodal culture of Solanecio biafrae. In *IOP Conference Series: Earth and Environmental Science*, *210*(1), 1-5. DOI:10.1088/1755-1315/210/1/012007

Bernstein, N., Gorelick, J., Koch, S. (2019). Interplay between chemistry and morphology in medical cannabis (*Cannabis sativa* L.). *Industrial Crops and Products*, *129*, 185-194. DOI:https://doi.org/10.1016/j.indcrop.2018.11.039

Blázquez, C., González-Feria, L., Alvarez, L., Haro, L., Casanova, M.LGuzman M. (2004). Cannabinoids inhibit the vascular endothelial growth factor pathway in gliomas. *Cancer Research*, *64* (16), 5617-23. DOI: https://doi.org/10.1158/0008-5472.CAN-03-3927

Bonini, S. A., Premoli, M., Tambaro, S., Kumar, A., Maccarinelli, G., Memo, M., & Mastinu, A. (2018). *Cannabis sativa*: A comprehensive ethnopharmacological review of a medicinal plant with a long history. *Journal of Ethnopharmacology*, *227*, 300-315. DOI: https://doi.org/10.1016/j.jep.2018.09.004

Caetano-Anollés, G., Favelukes, G., & Bauer, W. D. (1990). Optimization of surface sterilization for legume seed. Crop science, 30(3), 708-712. DOI:https://doi.org/10.2135/cropsci1990.0011183X003000030047x

Chahtane, H., Kim, W., & Lopez-Molina, L. (2017). Primary seed dormancy: a temporally multilayered riddle waiting to be unlocked. Journal of Experimental Botany, 68(4), 857-869. DOI: https://doi.org/10.1093/jxb/erw377

Daud, N. H., Jayaraman, S., & Mohamed, R. (2012). Methods Paper: An improved surface sterilization technique for introducing leaf, nodal and seed explants of Aquilaria malaccensis from field sources into tissue culture. *Aspac J. Mol Biol Biotechnol*, *20*, 55-58. https://www.researchgate.net/publication/286739042_Methods_paper_An_improved_surface_sterilization_technique_for_introducing_leaf_nodal_and_seed_explants_of_Aquilaria_malaccensis_from_field_sources_into_tissue_culture (Accessed 30.09.2021)

Ewel, J. J., Schreeg, L. A., & Sinclair, T. R. (2019). Resources for crop production: Accessing the unavailable. *Trends in Plant Science*, 24(2), 121-129. DOI: https://doi.org/10.1016/j.tplants.2018.10.008

FAO, 2019. http://www.fao.org/ (Accessed 30.09.2021)

Gamborg, O. L., Murashige, T., Thorpe, T. A., & Vasil, I. K. (1976). Plant tissue culture media. *In vitro*, *12*(7), 473-478. DOI: https://doi.org/10,1007/BF02796489

Geneve, R. L., Janes, E. W., & Kester, S. T. (2016, January). Cardinal temperatures and thermal time for seed germination of industrial hemp (Cannabis sativa)©. In Proceedings of the 2016 Annual Meeting of the International Plant Propagators' Society 1174 (pp. 325-330). DOI:10.17660/ActaHortic.2017.1174.65

Green, H., Broun, P., Cakmak, I., Condon, L., Fedoroff, N., Gonzalez-Valero, J., Graham I, Lewis, J., Moloney M, Oniang'o, R.K., SangingaN., Shewry, P. & Roulin, A. (2016). Planting seeds for the future of food. Journal of the science of food and agriculture, 96(5), 1409-1414. DOI:https://doi.org/10.1002/jsfa.7554

Greipsson, S. (2001). Effects of stratification and GA_3 on seed germination of a sand stabilising grass Leymus arenarius used in reclamation. *Seed. Science and Technology*, *29*, 1-10. DOI: https://doi.org/ No doi number published 2001. https://www.researchgate.net/profile/Sigurdur-Greipsson/publication/238114648_Effects_of_stratification_and_GA3_on_seed_germination_of_a_sand_stabilising_grass_Leymus_arenarius_used_in_reclamation/links/54a5d2eb0cf257a63608d7f5/Effects

-of-stratification-and-GA3-on-seed-germination-of-a-sand-stabilising-grass-Leymus-arenarius-used-in-reclamation.pdf (Accessed 30.09.2021)

Gribaudo I & Fronda A (1991). Effects of thidiazuron on grapevine axillary buds cultivated *In vitro*. *HortScience, 26*, 1083 DOI:https://doi.org/10.21273/HORTSCI.26.8.1083

Hall, J., Bhattarai, S. P., and Midmore, D. J. (2012). Review of flowering control in industrial hemp. J. Nat. Fibers 9, 23–36. DOI: https://doi.org/10.1080/15440478.2012.651848

Hanuš, L. O., Meyer, S. M., Muñoz, E., Taglialatela-Scafati, O., & Appendino, G. (2016). Phytocannabinoids: a unified critical inventory. *Natural Product Reports*, *33*(12), 1357-1392. DOI: https://doi.org/10.1039/C6NP00074F

Hesami, M., Naderi, R., & Tohidfar, M. (2019). Modeling and optimizing *In vitro* sterilization of chrysanthemum via multilayer perceptron-non-dominated sorting genetic algorithm-II (MLP-NSGAII). *Frontiers in plant science*, *10*, 282. DOI: https://doi.org/10.3389/fpls.2019.00282

Ines, M., Krunoslav, D., Vesna, T., Marija, V., Ankica, P., Zlatko, C., Boris, P., Zorica, J. (2013). *In vitro* sterilization procedures for micropropagation of 'Oblačinska'sour cherry. *Journal of Agricultural Sciences, Belgrade*, *58*(2), 117-126. DOI: https://doi.org/10.2298/JAS1302117M

ISTA (2021) https://www.seedtest.org/en/ista-rules-2019-_content---1--3410.html) (Accessed 30.09.2021)

Jovičić, D., Nikolić, Z., Sikora, V., Tamindžić, G., Petrović, G., Ignjatov, M., & Milošević, D. (2019). Comparison of methods for germination testing of Cannabis sativa seed. Ratarstvo i povrtarstvo/Field and *Vegetable* Crops Research, 56(3), 71-75. doi: 10.5937/ratpov56-21105

Kai, H. U., Li-Jun, Z., & Xue-mei, B. (2007). Analysis of the cause of contamination and explant sterilization in plant tissue culture. *Journal of Anhui Agricultural Sciences*, *35*(3), 680. DOI:https://en.cnki.com.cn/Article_en/CJFDTotal-AHNY200703024.htm

Khan, A. A. (1971). Cytokinins: permissive role in seed germination. *Science*, *171*(3974), 853-859. DOI: https://doi.org/ No doi number published 1971

Khan, P. S. V., Hausman, J. F., & Rao, K. R. (1999). Effect of agar, MS medium strength, sucrose and polyamines on *In vitro* rooting of Syzygium alternifolium. *Biologia Plantarum*, *42*(3), 333-340. : DOI: https://www.jstor.org/stable/1731340

Khawar, K. M., Sancak, C., Uranbey, S., & Ozcan, S. (2004). Effect of thidiazuron on shoot regeneration from different explants of lentil (*Lens culinaris* Medik.) via organogenesis. *Turkish Journal of Botany*, *28*(4), 421-426. DOI: https://doi.org/

Merve, G, & Orhan, K. (2020). Bitkisel Üretimde Yeni Bir Trend: Kenevir. *International Journal of Life Sciences and Biotechnology*, *4*(1-2), 2-2. DOI: https://doi.org/10.38001/ijlsb.789970

Mok, M.C. & Mok, D.W.S. (1985). The metabolism of (~4C)- thidiazuron in callus tissues of *Phaseolus lunatus*. *Physiologia Plantarum, 65*, 427-432 DOI: https://doi.org/10.1104/pp.73.3.796

Mok, M.C., Mok, D.W.S., Armstrong, D.J., Shudo, K., Isogai, Y. & Okamoto, T. (1982) Cytokinin activity of N-phenyI-N'- 1,2,3-thiadiazol-5-ylurea (thidiazuron). *Phytochemistry, 21*, 1509-1511. DOI: https://doi.org/10.1016/S0031-9422(82)85007-3 (Accessed 30.09.2021)

Nadjafia, F., Bannayan, M., Tabrizia, L. & Rastgoo, M. (2006). Seed germination and dormancy breaking techniques for Ferula gummosa and Teucrium polium. *Journal of Arid environments, 64*, 542-547. DOI: https://doi.org/10.1016/j.jaridenv.2005.06.009 (Accessed 30.09.2021)

Official Gazette Number: 29842 2016, Regulation on *Cannabis* Breeding and Control. https://www.resmigazete.gov.tr/(In Turkish) (Accessed 30.09.2021)

Official Gazette Number: 29842 2016, Sivas Governorship dated 06.01.2021 and E-27 145502-160.99-3718439. https://www.resmigazete.gov.tr/(In Turkish)

Owen, H. R., Wengerd, D., & Miller, A. R. (1991). Culture medium pH is influenced by basal medium, carbohydrate source, gelling agent, activated charcoal, and medium storage method. *Plant Cell Reports*, *10*(11), 583-586. DOI: https://www.10.1007/BF00232516

Paparella, S.; Araújo, S.S.; Rossi, G.; Wijayasinghe, M.; Carbonera, D.; Balestrazzi, A. (2015). Seed priming: State of the art and new perspectives. *Plant Cell Reports, 34*, 1281–1293. DOI: 10.1007/s00299-015-1784-y

Ritchie, S. & Gilroy, S. (1998). Gibberellins: regulating genes and germination. *New. Phytology,. 140*, 363-383. DOI: https://doi.org/ No doi number published 1998. https://www.cambridge.org/core/journals/new-phytologist/article/abs/gibberellins-regulating-genes-and-germination/50717265D3C671CA6C9A71F6395ECBAD (Accessed 30.09.2021)

Sánchez, C., de Ceballos, M. L., del Pulgar, T. G., Rueda, D., Corbacho, C., Velasco, G., Galve-Roperh I, Huffman J.W., y Cajal S.R. & Guzmán, M. (2001). Inhibition of glioma growth in vivo by selective activation of the CB2 cannabinoid receptor. *Cancer Research*, *61*(15), 5784-5789. DOI: https://doi.org/Published August 2001 (No Doi Number). https://cancerres.aacrjournals.org/content/61/15/5784.short

Sarwar, S., Zia, M., Rehman, R., Fatima, Z., Sial, R., & Chaudhary, M. (2009). *In vitro* direct regeneration in mint from different explants on half strength MS medium. *African Journal of Biotechnology*, *8*(18). https://www.researchgate.net/publication/253760732_In_vitro_direct_regeneration_in_mint_from_different_expplants_on_half_strength_MS_medium

Sattar, S., Hussnain, T., & Javaid, A. (2010). Effect of NaCl salinity on cotton (*Gossypium arboreum* L.) grown on MS medium and in hydroponic cultures. *The Journal of Animal & Plant Sciences*, *20*, 87-89. http://www.thejaps.org.pk/docs/20-2-2010/Sattar-et-al.pdf (Accessed 30.09.2021)

Schäfer, T. (2005). The Influence of Growing Factors and Plant Cultivation Methods on Biomass and Fibre Yield as Well as on Fibre Quality of Hemp (*Cannabis sativa* L.). *Journal of Natural Fibers*, *2*(1), 1-14. DOI: https://doi.org/10.1300/J395v02n01_01

Schumacher, A. G. D., Pequito, S., & Pazour, J. (2020). Industrial hemp fiber: A sustainable and economical alternative to cotton. *Journal of Cleaner Production*, *268*, 122180. DOI:https://doi.org/10.1016/j.jclepro.2020.122180

Seher, K., & Eren, O. (2020). Kenevir liflerinin eldesi, karakteristik ozellikleri ve tekstil endustrisindeki uygulamalari. *Mehmet Akif Ersoy Universitesi Fen Bilimleri Enstitusu Dergisi*, *11*(1), 108-123. DOI: https://doi.org/10.29048/makufebed.693406

Sera, B., Sery, M., Gavril, B., & Gajdova, I. (2017). Seed germination and early growth responses to seed pre-treatment by non-thermal plasma in hemp cultivars (*Cannabis sativa* L.). Plasma Chemistry and Plasma Processing, 37(1), 207-221. DOI: 10.1007/s11090-016-9763-9

Śledziński, P., Zeyland, J., Słomski, R., & Nowak, A. (2018). The current state and future perspectives of cannabinoids in cancer biology. *Cancer Medicine*, *7*(3), 765-775. DOI: https://doi.org/10.1002/cam4.1312

Snedecor, G.W. & Cochran, W.G. (1967) Statistical methods. 6th Edition, Ames, Iowa, the Iowa state University.

Spitzer-Rimon, B., Duchin, S., Bernstein, N., & Kamenetsky, R. (2019). Architecture and florogenesis in female Cannabis sativa plants. *Frontiers in plant science*, *10*, 350. DOI: https://doi.org/10.3389/fpls.2019.00350

Strzelczyk, M., Lochynska, M., & Chudy, M. (2021). Systematics and botanical characteristics of industrial hemp *Cannabis sativa* L. *Journal of Natural Fibers*, 1-23. DOI: https://doi.org/10.1080/15440478.2021.1889443

TSI, Crop Production Statistics, Turkey Statistical Institute, 2020. https://biruni.tuik.gov.tr/medas/(Accessed 30.09.2021)

Walck, J. L., Baskin, J. M., Baskin, C. C., & Hidayati, S. N. (2005). Defining transient and persistent seed banks in species with pronounced seasonal dormancy and germination patterns. Seed Science Research, 15(3), 189-196. DOI: https://doi.org/10.1079/SSR2005209