Soybean Seed Sterilization Methods in Relation to Germination and Early Growth

Metode sterilizacije sjemena soje u odnosu na klijavost i početni porast

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SOYBEAN SEED STERILIZATION METHODS IN RELATION TO GERMINATION AND EARLY GROWTH

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SUMMARY

The aim of this study was to evaluate the effect of different soybean seed sterilization methods on germination, contamination reduction, and early seedling growth. The experiment included 11 soybean cultivars and six sterilization treatments: control, 70 % ethanol, 5 % sodium hypochlorite, chloroform stabilized with amylene, chlorine gas, and UV-C treatment. The experiment was conducted under controlled laboratory conditions, with four replications per treatment. Standard germination, proportions of healthy, abnormal, infected, and dead seeds, root length, hypocotyl length, total seedling length, fresh mass, and dry mass were determined. The ANOVA showed a highly significant effect of genotype, sterilization treatment, and their interaction on all analyzed parameters ($p \leq 0.001$). Total germination was generally high, with an average of 88 %. The highest germination was recorded in chlorine gas and UV-C treatments, both reaching 94 %, while the lowest value was observed after chloroform-amylene treatment (78 %). The highest proportion of healthy seedlings was recorded after UV-C treatment (67 %), followed by chlorine gas (65 %) and 70 % ethanol (61 %). Chloroform-amylene showed the weakest overall performance, with the lowest percentage of healthy seedlings (43 %) and the highest percentage of abnormal seedlings (34%), although it also had the lowest rate of infected seedlings (1 %). In contrast, the control treatment had a lower proportion of healthy seedlings (45%) and higher levels of abnormal and infected seedlings. Generally, chlorine gas and UV-C treatments showed the most favorable effects on soybean seed germination and early seedling development, while chloroform-amylene was the least suitable treatment under the tested conditions, despite its strong reduction of infection.

Keywords: seed disinfection; surface treatment; seedlings; genotypes; early development, root, hypocotyl

INTRODUCTION

High-quality and healthy seeds represent the foundation of successful crop production. Seeds constitute a dynamic micro-ecosystem that harbors a complex community of epiphytic and endophytic microorganisms present on their surface and within internal tissues. This microbiota may play a key role in regulating germination, early plant growth and development, as well as in modulating plant responses to abiotic and biotic stress factors (Truyens et al., 2015; Nelson, 2018; Petrović et al., 2024).

Under controlled laboratory conditions, particularly in studies of germination and early plant ontogenesis, surface sterilization of seeds is a necessary prerequisite for reducing microbial contamination and ensuring reliable experimental results. However, the optimization of this procedure is methodologically challenging, as it requires a balance between effective elimination of epiphytic microbiota and preservation of seed structural integrity and physiological functionality. Insufficient sterilization may result in the persistence of microorganisms and subsequent contamination, whereas overly harsh treatments may cause damage to the seed coat and embryo, as well as disturbances in physiological processes involved in germination (Miché & Balandreau, 2001).

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The effectiveness of surface sterilization depends on the seed species, its morphological characteristics, the composition and abundance of associated microbiota, and the permeability of the seed coat. An important role is also played by the choice of sterilizing agent, its concentration, and treatment duration. The most commonly used chemical agents include ethanol (C_2H_5OH), sodium hypochlorite (NaOCl), hydrogen peroxide (H_2O_2), and mercuric chloride ($HgCl_2$). Ethanol is typically used as an initial treatment due to its rapid action, while NaOCl provides effective disinfection against a broad spectrum of microorganisms. Hydrogen peroxide represents a milder alternative, whereas the use of mercuric chloride is limited due to its high toxicity and adverse environmental impact (Bashan et al., 2012; Truyens et al., 2015; Karaca, 2024).

In recent years, alternative sterilization methods have been increasingly investigated with the aim of reducing the negative effects of chemical agents. One modern approach to seed pre-sowing treatment involves the application of ultraviolet (UV) radiation in combination with conventional treatments. This method includes exposing seeds to UV radiation, followed by treatment with standard chemical or biological control agents. UV pretreatment enables partial inactivation of pathogenic microflora and reduces the resistance of surviving microorganisms, thereby increasing the overall effectiveness of subsequent treatments (Vendin et al., 2023). Furthermore, in the sterilization of *Arabidopsis thaliana* seeds, fumigation with chlorine gas (Cl_2) has been used, demonstrating effective reduction of microbial contamination while maintaining seed germination capacity (Lindsey et al., 2017).

Despite the development of various sterilization methods, complete removal of microorganisms from seeds is often not achievable due to their presence in seed coat microcracks or internal tissues. Therefore, the choice of an optimal method depends on the objectives of the study and requires careful adjustment of sterilization conditions.

Given the importance of soybeans as a major source of plant proteins and oil, and as one of the most important field crops, the development of efficient and reliable seed surface sterilization methods is of great importance. Healthy seedlings and high germination are essential indicators of soybean seed quality, as they ensure uniform crop establishment, optimal plant density, and the successful production of high-quality seed. Germination testing of field crops is an important first step in evaluating seed quality and early seedling vigor, because it provides basic information on the ability of genotypes to establish uniform and healthy seedlings. Such information is also useful for further studies related to tolerance to abiotic and biotic stress conditions (Mangena, P., & Mokwala, 2019; Orkić et al., 2024; Lisjak et al., 2025).

Although seed sterilization prior to germination testing has been investigated in several crop species, including e.g., maize (Davoudpour et al., 2020), wheat (Aksoy, 2025), rice (Khamsen et al., 2016), cotton (Barampuram et al., 2014), legumes (Costa-Catala et al., 2024), eggplant (Kaur et al., 2014), and sweet pepper (Khah and Passam, 1992). The results show that sterilization efficiency depends on the disinfectant type, concentration, and treatment time, and that the best protocol is not the same for all species. Therefore, sterilization methods should be optimized for each crop separately.

Soybean seeds have specific structural and physiological characteristics that make the choice of sterilization method very important. Because soybean seeds absorb liquid rapidly, excessive exposure to sterilizing solutions may cause cotyledon separation and embryo damage. This can affect germination, seedling development, and the accuracy of test results. Therefore, an effective sterilization method for soybean seeds should reduce contamination and disease development while preserving seed viability and normal seedling growth. Thus, the aim of this study is to optimize the surface sterilization procedure of soybean seeds while maintaining high germination rates and normally developed seedlings.

MATERIALS AND METHODS

Seed Material

Germination testing was done at the Faculty of Agrobiotechnical Sciences Osijek (Croatia) in controlled conditions. For seed material, a total of 11 soybean cultivars developed by the Agricultural Institute Osijek were included in the analysis: *Ema*, *Ika*, *Korana*, *Lucija*, *OS Đurđica*, *OS Nevena*, *OS Zora*, *Sara*, *Sonja*, *Sunce*, and *Tena*.

Seed Sterilization Treatments

In this study, six different seed sterilization treatments were applied to soybean seeds prior to germination testing (Table 1). Control treatment was without any sterilization method and the sterilization treatments included both solution-based and gas-based methods, as well as ultraviolet-C (UV-C) sterilization.

Table 1. Soybean sterilization treatment

Tablica 1. Tretmani sterilizacije sjemena soje

Sterilization method / *Metoda sterilizacija*	**Treatment description** / *Opis tretmana*	**Treatment** / *Oznaka tretmana*
Control / *Kontrola*	No sterilization	C
Solution-based sterilization / *Sterilizacija u otopini*	70 % ethanol solution	Eth_70%
	5 % sodium hypochlorite solution	NaClO_5%
Gas-based sterilization / *Plinska sterilizacija*	Chloroform stabilized with amylene	Chl_amy
	Chlorine	Cl
Ultraviolet-C (UV-C) sterilization / *Sterilizacija ultraljubičastim C zračenjem (UV-C)*	Ultraviolet-C (UV-C)	UV-C

a) Solution-Based Seed Sterilization

A 70% ethanol solution was prepared by diluting 96 % ethanol with distilled water. The required volume of 96% ethanol was calculated using the dilution equation: $C_1V_1=C_2V_2$; where C_1 represents the initial ethanol concentration, V_1 the volume of 96 % ethanol required, C_2 the desired ethanol concentration, and V_2 the final volume of the prepared solution. In this study, C_1 = 96 % and C_2 = 70 %.

A 5 % sodium hypochlorite (NaClO) solution was prepared by diluting commercial bleach (Cekina, containing 26.67 % sodium hypochlorite) with distilled water. For the preparation of 1 L of the working solution, 187.5 mL of commercial bleach was mixed with 812.5 mL of distilled water.

Seeds of each soybean genotype were immersed in the prepared 70 % ethanol or 5 % sodium hypochlorite solution for 2 minutes. After sterilization, the seeds were placed between sterile cellulose paper towels and dried for 24 hours. Following drying, the seeds were sown for germination testing.

b) Surface Sterilization of Soybean Seeds by Gas Fumigation

Gas sterilization of soybean seeds was performed using chloroform stabilized with amylene (Fisher Chemical, Fisher Scientific, USA) in a vacuum desiccator. For each soybean genotype, 150 g of seeds were weighed and placed in 250 mL labeled glass beakers. Moist sterile cellulose paper towels were placed at the bottom of the vacuum desiccator to maintain humidity during the sterilization treatment. The glass beakers containing the seeds were then arranged inside the desiccator according to genotype labels.

An open glass beaker containing chloroform stabilized with amylene was positioned in the center of the desiccator, and boiling stones A (Pöllath Labor Technologie, Germany) were added to promote uniform boiling. The vacuum desiccator was placed in a fume hood and connected to a portable membrane vacuum pump. Vacuum was applied until the chloroform began to boil, allowing chloroform vapor to fill the desiccator chamber. After boiling was achieved, the vacuum pump was turned off, and the seeds were left in the closed vacuum desiccator for 24 h in an atmosphere of gaseous chloroform stabilized with amylene. Following gas sterilization, the seeds were removed from the desiccator and used for germination testing.

Surface sterilization of soybean seeds by chlorine gas fumigation was also done in a vacuum desiccator. For each cultivar, 170 g of seeds were placed in 250 mL glass beakers and transferred to a desiccator within a fume hood. Chlorine gas was generated by mixing 20 mL of 23 % (w/v) sodium hypochlorite (NaOCl) with 5.64 mL of 12 M hydrochloric acid (HCl) in a 100 mL beaker positioned at the center of the desiccator. The reaction produced chlorine bubbles, which, due to their low solubility in concentrated NaOCl, were released from solution into the desiccator. Immediately following the addition of HCl, the desiccator was sealed, and the seeds were exposed to the gas for 20 h. After sterilization, the desiccator was opened, and the seeds were aerated for one hour to remove residual gas before being prepared for germination. The amount of HCl required to achieve a 5% chlorine concentration was calculated using the equation, as described by Lindsey et al. (2017). The net volume of the desiccator, calculated as 16 L (total volume of 18 L minus 2 L seed displacement), was converted to milliliters and applied as:

$$VHCl = \left(\frac{\%Cl_2 \text{ x } V_{dessicator}}{149.4 \text{ x } (100-\%Cl_2)}\right) mL$$

c) Ultraviolet (UV) Sterilization

Ultraviolet-C (UV-C) soybean seed sterilization was performed in a laminar (Iskra Pio, Slovenia) flow cabinet. For each soybean genotype, 150 g of seeds were weighed and placed in open glass Petri dishes with a diameter of 200 mm. The seeds were evenly distributed in a single layer, without overlapping, to ensure direct exposure to UV-C light.

The open Petri dishes containing the seeds were placed under UV-C lamps inside the laminar flow cabinet. Sterilization was carried out by exposing the seeds to UV-C light for 24 h. Following UV-C treatment, the seeds were used for germination testing.

Soybean Sowing and Measurements

Fifty seeds of each cultivar were sown on filter paper using the between-paper method according to ISTA 2026. The filter paper, 80 g/m², dimension 290 × 290 mm (Munktell, Germany), was soaked with 30 ml of distilled water. The seeds were placed between rolled filter papers, inserted into labeled plastic bags, and positioned vertically in an incubator chamber (Memmert ICH260C) in dark conditions at a temperature of 25 °C. All sterilization treatments and the control treatment were sown in 4 replications.

Seedlings Evaluation Parameters

Following germination testing, on the 8th day, seedlings were evaluated by determining the percentages of normal seedlings, abnormal seedlings, and dead seeds, in accordance with ISTA Rules (2026). Although infected seedlings are not classified as a separate category according to ISTA, they were recorded separately in this study in order to assess the effect of sterilization treatments on disease development in soybean seedlings. Standard germination was also calculated. Furthermore, after germination, morphological parameters of soybean seedling were determined: root, hypocotyl, and total length (cm), fresh mass – FM (g per seedling), and dry mass – DM (g per seedling). Based on these values, seedlings' water content and dry matter content were calculated (DM/FM × 100). Water content (%) was calculated as: (FM - DM) / FM × 100. Dry matter content (%) was calculated as: DM/FM × 100.

Biometrical Approach

Analysis of variance (ANOVA) was performed using SAS Enterprise Guide 7.1 software. When the F-test indicated statistically significant differences, mean comparisons were conducted using the least significant difference (LSD) test. Statistical significance was determined at the $p \leq 0.05$ level. To show interaction between sterilization treatment and genotype, the heat maps were generated in the Chi Plot system (https://www.chiplot.online/).

RESULTS AND DISCUSSION

The ANOVA results (Table 2) showed that all tested factors had a highly significant effect on total germination and early growth parameters of soybean seedlings. Genotype significantly influenced total germination, root length, hypocotyl length, total seedling length, fresh mass, dry mass, water content, and dry matter content ($p \leq 0.001$). This indicates that the tested soybean genotypes differed strongly in their germination ability and early seedling development.

Sterilization also had a highly significant effect on all analyzed parameters ($p \leq 0.001$). This means that the applied sterilization treatments influenced not only germination, but also seedling growth and biomass accumulation.

The interaction between genotype and sterilization was also highly significant for all parameters ($p \leq 0.001$). Therefore, both genotype and sterilization treatment should be considered when selecting the most suitable treatment for soybean seed germination and early seedling growth.

The results show that sterilization treatments had different effects on total germination and early growth of soybean seedlings (Figure 1 a-d). Total germination was generally high in all treatments, with an average value of 88 %. The highest total germination was recorded in the CI and UV-C treatments (Figure 1a), both with 93 %. NaClO_5% also showed a high germination value of 88 %, while the control had 87 %, and Eth_70% had 86 %. The lowest total germination was observed in the Chl_amy treatment, with 79 %.

Table 2. Summary of variance analysis – significance of the F-test of the morphological and mass-related parameters of soybean seedlings

Tablica 2. Sažetak analize varijance — značajnost F-testa za morfološke pokazatelje i pokazatelje povezane s masom klijanaca soje

Source of variation/ *Izvor varijacije*	**Degrees of freedom** / *Stupnjevi slobode*	**Standard germination** / *Ukupna klijavost*	**Root length** / *Dužina korijena*	**Hypocotyl length** / *Dužina hipokotila*	**Total length** / *Ukupna dužina*	**Fresh mass** / *Svježa masa*	**Dry mass** / *Suha masa*	**Water content (%)** / *Sadržaj vode (%)*	**Dry matter content (%)** / *Udio suhe tvari (%)*
Genotype / *Genotip*	10	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Sterilization / *Sterilzacija*	5	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Genotype × Sterilization / *Genotip × Sterilizacija*	50	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

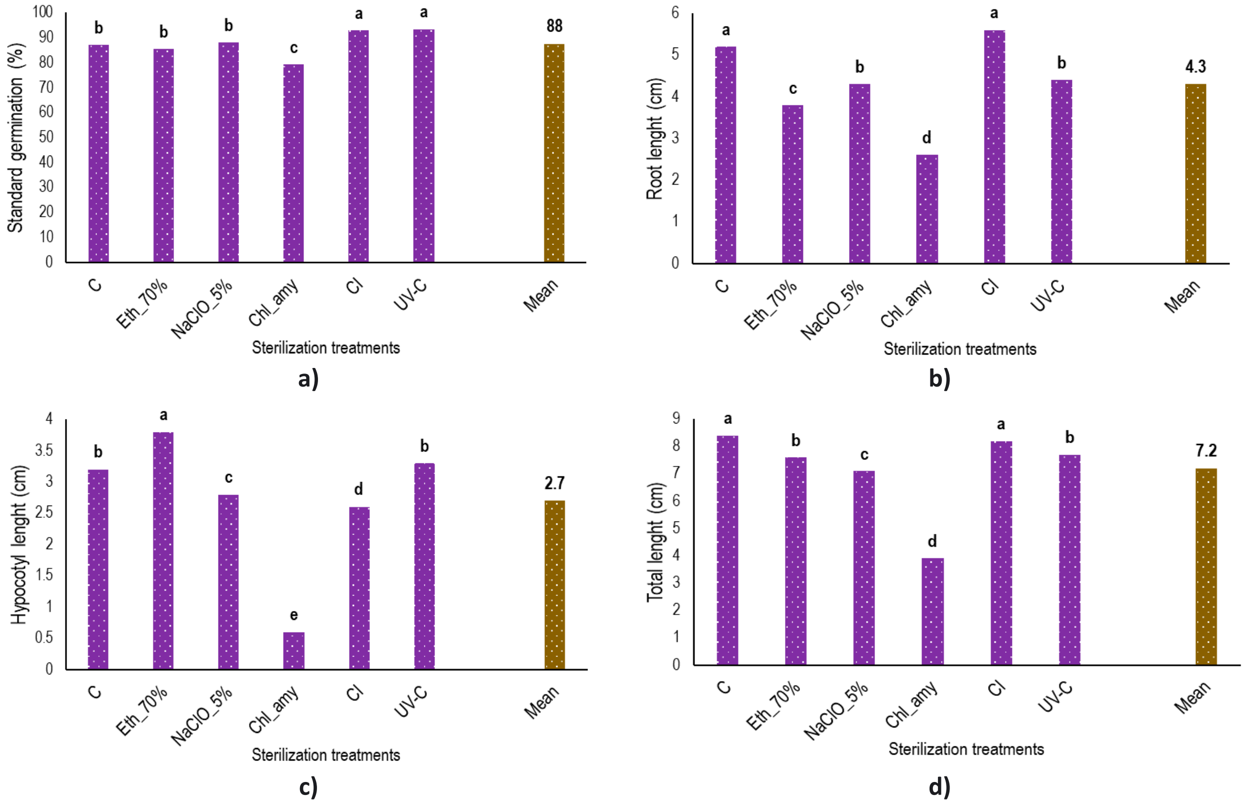

Figure 1. Effect of sterilization treatments on standard germination, root length, hypocotyl length, and total length of soybean seedlings. Different letters represent significant differences at p ≤ 0.05.

Grafikon 1. Utjecaj tretmana sterilizacije na ukupnu klijavost, dužinu korijena, dužinu stabljike i ukupnu dužinu klijanaca soje. Različita slova označuju statistički značajne razlike pri p ≤ 0,05.

Root length significantly differed among treatments (Figure 1 b). The longest roots were recorded in the Cl treatment, with 5.6 cm, followed by the control treatment with 5.2 cm. UV-C and NaClO_5% had moderate root length values, 4.4 cm and 4.3 cm, respectively. Treatment Eth_70% resulted in reduced soybean seedlings root length to 3.8 cm, while the shortest roots were found in the Chl_amy treatment, with only 2.6 cm. This indicates that Chl_amy had the strongest negative effect on root development. Hypocotyl length (Figure 1 c) was highest in the Eth_70% treatment, with 3.8 cm, followed by UV-C with 3.3 cm and the control with 3.2 cm. NaClO_5% and Cl had lower values, 2.8 cm and 2.6 cm, respectively. The lowest hypocotyl length was again recorded in the Chl_amy treatment, with 0.6 cm. A similar trend was observed for total seedling length (Figure 1 d). The highest total length was recorded in the control treatment, with 8.4 cm, followed by Cl with 8.2 cm and UV-C with 7.7 cm. Eth_70% also showed relatively good seedling length, with 7.6 cm. NaClO_5% had a lower value of 7.1 cm, while Chl_amy had the lowest total seedling length, only 3.9 cm. The positive effect of chlorine gas treatment is supported by Lindsey et al. (2017), who showed that chlorine gas sterilization can provide efficient seed surface sterilization while maintaining high germination. In this study, Cl treatment resulted in one of the highest germination values and the greatest root length. The relatively good practice of ethanol and NaClO is consistent with Gilbert et al. (2023), who reported that ethanol and sodium hypochlorite are effective seed disinfestation treatments that can reduce fungal growth without strongly reducing germination.

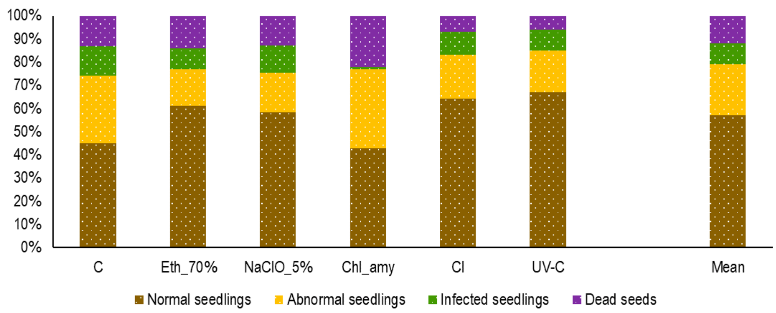

Figure 2. Effect of sterilization treatments on the percentage of normal, abnormal, and infected soybean seedlings and dead seeds.

Grafikon 2. Utjecaj tretmana sterilizacije na udio zdravih klijanaca, abnormalnih klijanaca, zaraženih klijanaca i mrtvoga sjemena soje.

Treatment with UV-C (Figure 2) showed the highest percentage of normal seedlings (67 %), followed by Cl treatment (65 %) and Eth_70% (61 %). The lowest percentage of normal seedlings was observed in Chl_amy (43 %), which also had the highest proportion of abnormal seedlings (34 %) and non-seedlinged seeds (22 %). The lowest level of infected seedlings was recorded in Chl_amy (1 %), suggesting strong suppression of infection; however, this treatment also negatively affected normal seedlinging. Overall, UV-C appears to be the most effective treatment, as it produced the highest number of normal seedlings while maintaining relatively low infection and non-seedlinging levels. Davoudpour et al. (2020) emphasized that efficient seed surface sterilization should reduce microbial contamination without negatively affecting seedling development. This agrees with the present results, where some treatments improved germination but did not always improve root, hypocotyl, or total seedling length.

Interaction between genotype and sterilization treatment for germination and initial growth parameters confirms that the efficiency of sterilization treatments depended on genotype (Figure 4 a-f). The results indicate that soybean genotypes responded differently to the applied sterilization treatments. For total germination, the highest values were mainly observed after Cl and UV-C treatments, especially in *Korana*, *Sara*, and *Lucija*, while the lowest values were recorded in *Sara* and *OS Đurđica* treated with Chl_amy (Figure 4 a). Root length also varied strongly among genotypes and treatments. The highest root length was recorded in *Lucija* and *Sunce* under the Cl treatment, while the lowest values were mostly found after Chl_amy treatment (Figure 4 b).

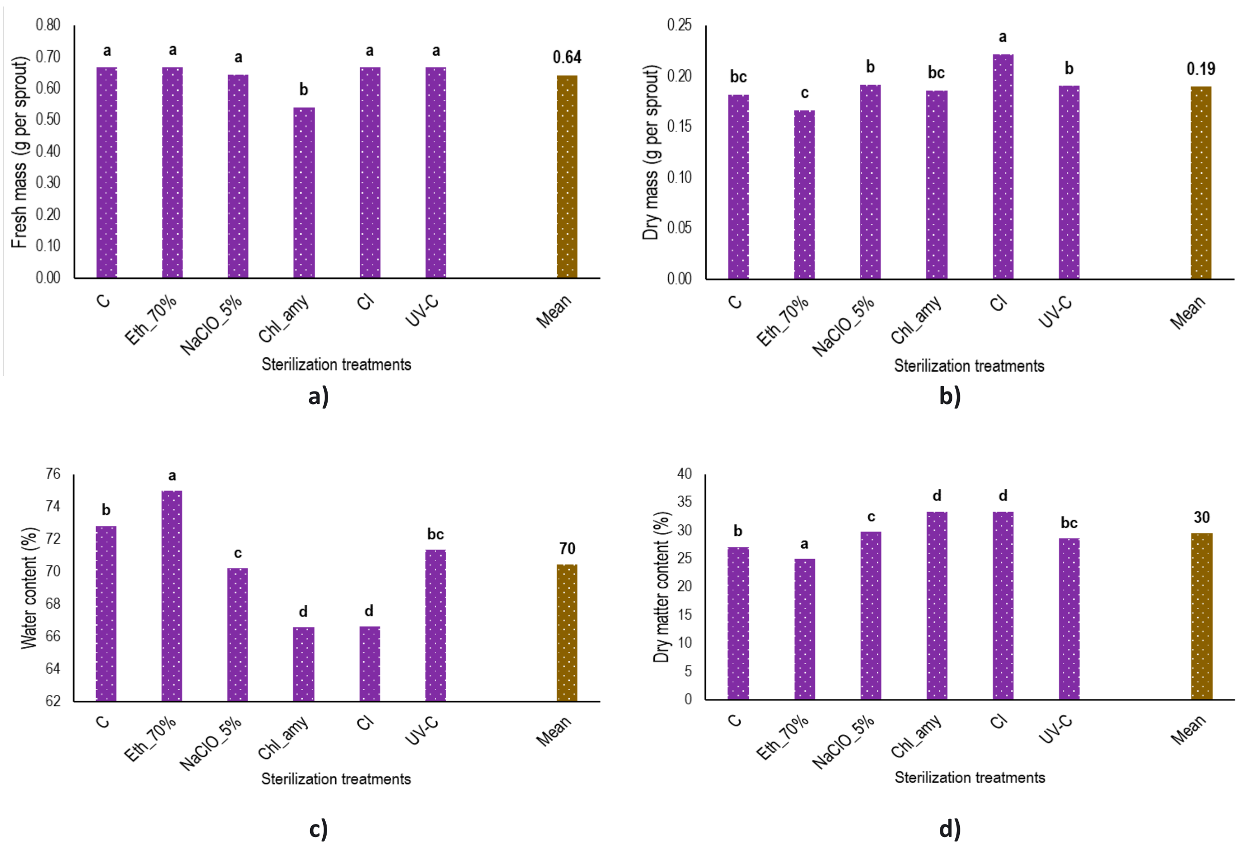

Figure 3. Effect of sterilization treatments on fresh mass, dry mass, water content, and dry matter content of soybean seedlings. Different letters represent significant differences at $p \leq 0.05$

Grafikon 2. Utjecaj tretmana sterilizacije na svježu masu, suhu masu, sadržaj vode i udio suhe tvari klijanaca soje. Različita slova označuju statistički značajne razlike pri $p \leq 0,05$

Hypocotyl length showed a different response pattern. Higher values were observed in several genotypes treated with Eth_70%, control and UV-C, while Chl_amy generally resulted in the shortest hypocotyls (Figure 4 c). A similar trend was found for total seedling length, where the highest values were recorded in *Sunce* under Cl treatment, *Sara* under UV-C, and Ika under Eth_70%, while Chl_amy produced the lowest total seedling length in most genotypes (Figure 4 d). Fresh matter was highest in *Sara* treated with Chl_amy and *OS Đurđica* in the control treatment, but these values should be interpreted together with germination and growth results (Figure 4e). Dry matter showed the highest value in *Sara* under Chl_amy, while the lowest dry matter values were recorded in Sonja treated with Chl_amy (Figure 4 f).

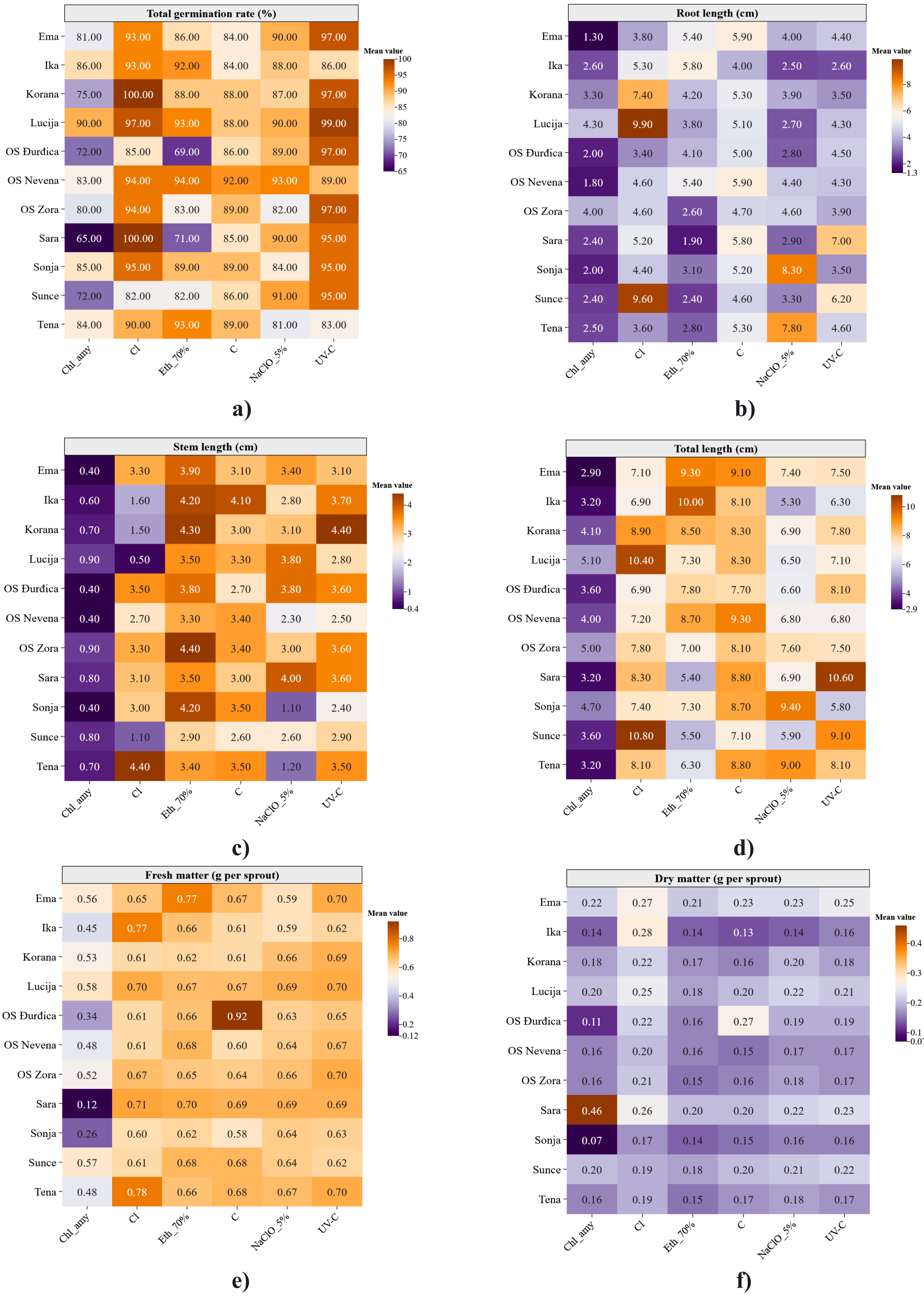

Figure 4. Interaction effect of genotype and sterilization treatment on early growth parameters of soybean seedlings. The color intensity represents the mean value.

Grafikon 4. Interakcijski učinak genotipa i tretmana sterilizacije na pokazatelje ranoga porasta klijanaca soje. Intenzitet boje prikazuje srednju vrijednost.

Similar results were reported by Costa-Catala et al. (2024), who showed that seed disinfection treatments can affect germination in legumes, with sodium hypochlorite improving germination in some species. In the present study, NaClO_5 % also maintained a high germination rate, but its effect on seedling growth depended on the analyzed parameter. The favorable effect of UV-C observed in this study is in agreement with Vendin et al. (2023), who reported that UV radiation can effectively reduce microbial contamination on the surface of soybean seeds. In the present study, UV-C resulted in high total germination and the highest percentage of normal seedlings.

Recent studies further support the observation that seed sterilization protocols must be optimized according to crop species, genotype, seed structure, and the intended experimental purpose. Parnell et al. (2024) reported that seed germination and fungal contamination varied among maize genotypes and seed sources, concluding that both variety and seed origin should be considered when optimizing sterilization protocols. This is in agreement with the present study, where the significant genotype × sterilization interaction showed that soybean cultivars differed in their response to the applied treatments. Similarly, Moumni et al. (2023), in a review of recent seed treatment methods for the control of seedborne pathogens, emphasized that physical and chemical seed treatments may reduce pathogen incidence, but their efficiency depends strongly on treatment duration, dose, crop species, seed coat characteristics, and pathogen location. This supports the present results, where treatments such as Cl and UV-C had favorable effects on germination and seedling development, while Chl_amy strongly reduced infection but negatively affected germination and early growth. Shen and Singh (2024) also highlighted UV treatment as a promising non-thermal method for seed and sprout disinfection, but noted that UV efficiency depends on dose and exposure conditions and may either promote or inhibit sprouting. This agrees with the high proportion of normal seedlings obtained after UV-C treatment in the present study and confirms the importance of selecting treatment conditions that reduce contamination without impairing seedling vigor. In addition, recent findings by Johnston-Monje and Martínez (2025) showed that seed surface sterilization can alter root-associated microbiomes and plant phenotype, indicating that sterilization may influence not only contamination levels but also subsequent seedling development. Therefore, the present results are consistent with recent literature showing that the most suitable sterilization method should be selected by considering both disinfection efficiency and seedling performance, rather than contamination reduction alone

CONCLUSION

In general, the results suggest that Cl and UV-C treatments were the most effective for improving total germination, while Cl also had the best effect on root length. The control treatment showed the highest total seedling length, which indicates that some sterilization treatments may improve germination but not necessarily increase seedling growth. Cl and UV-C treatments generally showed favorable effects on germination and some growth parameters. Chloroform-amylene showed the weakest results for all measured growth parameters and also had the lowest germination percentage. Therefore, this treatment appears to be the least suitable for soybean seed sterilization under the tested conditions. These findings indicate that the selection of an appropriate sterilization method is important not only for reducing seedborne infection but also for preserving seed viability and normal seedling development. The results also confirmed that sterilization efficiency is genotype-dependent, suggesting that the same treatment may not be equally suitable for all soybean cultivars. Although chloroform-amylene strongly reduced the proportion of infected seedlings, its negative effect on germination and seedling growth limits its practical application. In contrast, chlorine gas and UV-C treatments provided a better balance between high germination, reduced infection, and favorable early seedling development. Therefore, these methods may be considered promising approaches for soybean seed sterilization in laboratory germination testing and early growth studies. Further research should include optimization of treatment duration and intensity in order to improve sterilization efficiency while minimizing possible adverse effects on seedling vigor.

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REFERENCES

1. Aksoy, B. T. (2025). Surface Disinfection of Bread Wheat Seeds for in vitro Germination. *Bilecik Şeyh Edebali Üniversitesi Fen Bilimleri Dergisi*, *12*(1), 301-310. https://doi.org/10.35193/bseufbd.1559218
2. Barampuram, S., Allen, G., & Krasnyanski, S. (2014). Effect of various sterilization procedures on the *in vitro* germination of cotton seeds. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *118*(1), 179-185. https://doi.org/10.1007/s11240-014-0472-x
3. Bashan, Y., de-Bashan, L. E., Prabhu, S. R., & Hernandez, J. P. (2012). Advances in plant growth-promoting bacterial inoculant technology. Plant and Soil, 378, 1–33. https://doi.org/10.1007/s11104-013-1956-x
4. Costa-Catala, J., Bori, J., Veciana-Nogués, M. T., Latorre-Moratalla, M. L., Vidal-Carou, M. C., & Comas-Basté, O. (2024). Influence of seed disinfection treatments on the germination rate and histamine-degrading activity of legume sprouts. *Foods*, *13*(24), 4105. https://doi.org/10.3390/foods13244105
5. Davoudpour, Y., Schmidt, M., Calabrese, F., Richnow, H. H., & Musat, N. (2020). High resolution microscopy to evaluate the efficiency of surface sterilization of *Zea Mays* seeds. *Plos one*, *15*(11), e0242247. https://doi.org/10.1371/journal.pone.0242247
6. Davoudpour, Y., Schmidt, M., Calabrese, F., Richnow, H. H., & Musat, N. (2020). High resolution microscopy to evaluate the efficiency of surface sterilization of *Zea mays* seeds. *PLOS ONE, 15*(11), e0242247. https://doi.org/10.1371/journal.pone.0242247
7. Gilbert, G. S., Diaz, A., & Bregoff, H. A. (2023). Seed disinfestation practices to control seed-borne fungi and bacteria in home production of sprouts. Foods, 12(4), 747. https://doi.org/10.3390/foods12040747
8. https://www.chiplot.online/ (Accessed on 30 April 2026)
9. International Seed Testing Association. (2026). International rules for seed testing 2026. International Seed Testing Association.
10. Johnston-Monje, D., Martínez, J. P. (2025). Seed surface sterilization can alter root microbiomes, increase endophyte diversity and enhance plant growth. *Applied Sciences, 15*(17), 9545. https://doi.org/10.3390/app15179545
11. Karaca, G. H., Deveci, H., Albayrak, I., Baydar, N. G. (2024). Effects of different surface sterilization protocols on fungal load and germination of black henbane seeds. *Mediterranean Agricultural Sciences*, 37(3), 115-120. https://doi.org/10.29136/mediterranean.1530967
12. Kaur, M., Dhatt, A. S., & Sidhu, A. S. (2014). Effect of seed disinfectants on in vitro seed germination and seedling development in eggplant. *Journal of Horticultural Sciences*, *9*(1), 61-65. https://doi.org/10.24154/jhs.v9i1.224
13. Khah, E. M., & Passam, H. C. (1992). Sodium hypochlorite concentration, temperature, and seed age influence germination of sweet pepper. *HortScience*, *27*(7), 821-823.
14. Khamsen, N., Onwimol, D., Teerakawanich, N., Dechanupaprittha, S., Kanokbannakorn, W., Hongesombut, K., & Srisonphan, S. (2016). Rice (*Oryza sativa* L.) seed sterilization and germination enhancement via atmospheric hybrid nonthermal discharge plasma. *ACS applied materials & interfaces*, *8*(30), 19268-19275. https://doi.org/10.1021/acsami.6b04555.
15. Lindsey, B. E. 3rd., Rivero, L., Calhoun, C. S., Grotewold, E., & Brkljacic, J. (2017). Standardized Method for High-throughput Sterilization of Arabidopsis Seeds. *Journal of visualized experiments: JoVE*, (128), 56587. https://doi.org/10.3791/56587
16. Lisjak, M., Ocvirk, D., Špoljarević, M., Teklić, T., Liović, I., Špoljarić Marković, S., ... Mijić, A. (2025). Učinak primiranja sjemena sumporovodikom na klijanje i biokemijske pokazatelje sušnog stresa kod klijanaca suncokreta. Poljoprivreda, 31 (1), 1-12. https://doi.org/10.18047/poljo.31.1.1
17. Mangena, P., & Mokwala, P. W. (2019). The influence of seed viability on the germination and in vitro multiple shoot regeneration of soybean (*Glycine max* L.). *Agriculture, 9*(2), 35. https://doi.org/10.3390/agriculture9020035
18. Miché, L., Balandreau, J. (2001). Effects of rice seed surface sterilization. *Applied and Environmental Microbiology*, 67(11), 5594–5600. https://doi.org/10.1128/AEM.67.7.3046-3052.2001
19. Moumni, M., Brodal, G., & Romanazzi, G. (2023). Recent innovative seed treatment methods in the management of seedborne pathogens. *Food Security, 15*(5), 1365–1382. https://doi.org/10.1007/s12571-023-01384-2
20. Nelson, E. B. (2018). The seed microbiome. Annual Review of Phytopathology, 56, 361–384. https://doi.org/10.1111/1751-7915.14352
21. Orkić, V., Grubišić–Šestanj, S., Ravnjak, B., Rebekić, A., Petrović, S., Vila, S., ... Kujundžić, S. (2024). Prijažetveno proklijavanje kod različitih kultivara pšenice. *Poljoprivreda, 30* (1), 21-27. https://doi.org/10.18047/poljo.30.1.3
22. Parnell, J. J., Pal, G., Awan, A., Vintila, S., Houdinet, G., Hawkes, C. V., Balint-Kurti, P. J., Wagner, M. R., & Kleiner, M. (2024). Effective seed sterilization methods require optimization across maize genotypes. *Phytobiomes Journal, 8*(4), 418–424. https://doi.org/10.1094/PBIOMES-12-23-0137-R
23. Petrović, E., Vrandečić, K., Ćosić, J. i Godena, S. (2024). Chemical Control of Olive Fungal Diseases: Strategies and Risks. *Poljoprivreda, 30* (1), 44-53. https://doi.org/10.18047/poljo.30.1.6
24. SAS Institute Inc. (2014). SAS Enterprise Guide (Version 7.1). SAS Institute Inc.
25. Shen, M.-H., & Singh, R. K. (2024). Elicitation and disinfection during sprout production using ultraviolet radiation and hydrogen peroxide: A review. *Trends in Food Science & Technology, 147*, 104447. https://doi.org/10.1016/j.tifs.2024.104447
26. Truyens, S., Weyens, N., Cuypers, A., Vangronsveld, J. (2015). Bacterial seed endophytes. Plant and Soil, 405, 1–13. https://doi.org/10.3390/microorganisms5040070
27. Vendin, S., Strakhov, V., & Manuilenko, A. (2023). Results of studies on the application of UV radiation for disinfecting the surface of soybean seeds from pathogenic microflora. *BIO Web of Conferences, 67*, 02027. https://doi.org/10.1051/bioconf/20236702027

METODE STERILIZACIJE SJEMENA SOJE U ODNOSU NA KLIJAVOST I POČETNI PORAST

SAŽETAK

Cilj ovoga istraživanja bio je procijeniti utjecaj različitih metoda sterilizacije sjemena soje na klijavost i početni rast klijanaca. U istraživanje je bilo uključeno 11 sorata soje i šest tretmana sterilizacije: kontrola, 70 % etanol, 5 % natrijev hipoklorit, kloroform stabiliziran amilenom, plinoviti klor i UV-C tretman. Pokus je proveden u kontroliranim laboratorijskim uvjetima, u četiri ponavljanja po tretmanu. Određeni su ukupna klijavost, udio normalnih, abnormalnih, bolesnih klijanaca i mrtvoga sjemena, potom dužina korijena, dužina hipokotila, ukupna dužina klijanca, svježa masa i suha masa. ANOVA je pokazala visoko značajan utjecaj genotipa, tretmana sterilizacije i njihove interakcije na sve analizirane pokazatelje ($p \leq 0,001$). Ukupna klijavost bila je općenito visoka, prosječno 88 %. Najviša klijavost utvrđena je kod tretmana klornim plinom i UV-C zračenjem, pri čemu su oba tretmana dosegnula 94 %, dok je najniža vrijednost utvrđena nakon tretmana kloroform stabiliziranoga amilenom (78 %). Najveći udio normalnih klijanaca soje utvrđen je nakon tretmana UV-C zračenjem (67 %), zatim kod tretmana klornim plinom (65 %) i 70 %-tnim etanolom (61 %). Kloroform stabiliziran amilenom pokazao je najslabiji ukupni učinak, s najnižim udjelom zdravih klica (43 %) i najvišim udjelom abnormalnih klica (34 %), iako je istovremeno imao najniži udio zaraženih klica (1 %). U usporedbi s tim, kontrolni tretman imao je niži udio normalnih klijanaca (45 %) te veći udio abnormalnih i zaraženih klijanaca. Općenito, tretmani klornim plinom i UV-C zračenjem pokazali su najpovoljniji učinak na klijavost sjemena soje i rani razvoj klijanaca, dok se kloroform stabiliziran amilenom pokazao najmanje prikladnim tretmanom u ispitivanim uvjetima, unatoč izraženomu smanjenju udjela bolesnih klijanaca soje.

Ključne riječi: dezinfekcija sjemena, površinski tretman, genotipovi, početni razvoj, korijen, hipokotil

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