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Araştırma Makalesi - Research Article

Surface Disinfection of Bread Wheat Seeds for *in vitro* Germination

Ekmeklik Buğday Tohumlarının *in vitro* Çimlenmesinde Yüzey Dezenfeksiyonu

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ABSTRACT

In the process of optimizing seed germination, *in vitro* seed sterilization is complex due to the influence of many factors (e.g., genotype, disinfectants, temperature, light, and application time). This study compared the efficacy of both sodium hypochlorite and hydrogen peroxide disinfectants at three concentrations (5%, 10%, 20%) and three treatment times (5, 10, 15 minutes). These are the most used disinfectants for *in vitro* seed sterilization on wheat. Additionally, the germination and contamination rates of surface disinfection in sterile petri dishes were examined as the initial step for molecular and breeding studies in wheat. Upon examining the results of a total of 19 different disinfection treatment combinations, the findings of this study highlight the significance of a 20% sodium hypochlorite concentration with a 15-minute treatment time as the optimal disinfection method minimizing contamination in the *in vitro* Murashige and Skoog medium. Method number 8 (20% NaClO – 10 min), which showed the highest germination percentage at 95%, was determined based on the germination results obtained on sterile filter papers in petri dishes. These results provide valuable tools to aid with molecular and breeding studies related to wheat cultivation and improvement of surface seed disinfection.

Keywords- Contamination, Disinfection, Germination, Wheat Seed

ÖZ

Tohum çimlenmesini optimize etme sürecinde, *in vitro* tohum sterilizasyonu birçok faktörün (örneğin genotip, dezenfektanlar, sıcaklık, ışık ve uygulama süresi) etkisi nedeniyle karmaşıktır. Bu çalışmada, sodyum hipoklorit ve hidrojen peroksit dezenfektanlarının üç konsantrasyonda (%5, %10, %20) ve üç işlem süresinde (5, 10, 15 dakika) etkinliği karşılaştırılmıştır. Bunlar, buğdayda *in vitro* tohum sterilizasyon için en çok kullanılan dezenfektanlardır. Ayrıca, buğdayda moleküler ve ıslah çalışmaları için ilk adım olarak steril petri kaplarında yüzey dezenfeksiyonunun çimlenme ve kontaminasyon oranları incelenmiştir. Toplam 19 farklı dezenfeksiyon işlem kombinasyonunun sonuçları incelendiğinde, bu çalışmanın bulguları, *in vitro* Murashige ve Skoog besiyerinde kontaminasyonu en aza indiren optimum dezenfeksiyon yöntemi olarak 15 dakikalık işlem süresine sahip %20'lik bir sodyum hipoklorit konsantrasyonunun önemini vurgulamaktadır. En yüksek çimlenme yüzdesini %95 olarak gösteren yöntem numarası 8 (%20 NaClO – 10 dk), petri kaplarındaki steril filtre kağıtlarında elde edilen çimlenme sonuçlarına dayanarak belirlenmiştir. Bu sonuçlar, buğday yetiştiriciliği ve yüzey tohum dezenfeksiyonunun iyileştirilmesiyle ilgili moleküler ve ıslah çalışmalarına yardımcı olmak üzere karşılaştırmalı bilgiler sunmaktadır.

Anahtar Kelimeler- Kontaminasyon, Dezenfeksiyon, Çimlenme, Buğday Tohumu

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I. INTRODUCTION

Wheat (*Triticum aestivum* L.) began to be cultivated approximately ten thousand years ago, and today it is the most cultivated and consumed grain in the world [1,2]. In addition, wheat, which is a significant source of protein, is exposed to biotic (fungi, bacteria, etc.) and abiotic (salt, cold, etc.) stresses that can lead to serious yield losses in its production [3,4]. From past to present, many breeding studies have been carried out on wheat, aiming to increase the quality and productivity. In this regard, in addition to breeding studies, *in vitro* micropropagation techniques are used to accelerate the germination and growth of wheat, shorten the development period, and obtain more productive seeds.

Traditional germination methods face various limitations, including seasonal production, labor demands, time consumption, pest infestation, and disease susceptibility, making large-scale plant production challenging. Therefore, to overcome these limitations and sustain wheat production, the application of new approaches such as tissue culture is considered a desirable alternative. *In vitro* culture techniques are employed in numerous fields: rapid plant production [5–7], developmental biology [8,9], secondary metabolite production [10–12], and bioenergy production [13]. Moreover, *in vitro* culture systems offer significant advantages, such as investigating seed germination physiology, controlling environmental conditions, and preserving genetic resources through cryopreservation [14,15]. In this regard, *in vitro* techniques like tissue culture are significant tools for optimizing germination processes and supporting plant breeding efforts. These methods provide a more efficient and controlled environment, surpassing the limitations of traditional germination methods, and thereby facilitating large-scale plant production and genetic diversity preservation.

Seeds from field or greenhouse environments are exposed to many contaminants such as bacteria, fungi, yeast, and viruses [8,16,17]. Effective surface sterilization of seeds or explants intended for culture initiation constitutes the initial step in both *in vitro* propagation and other molecular or classical breeding studies [18]. It is worth noting that while disinfection may be deemed irrelevant in classical breeding studies, *in vitro* technologies necessitate meticulous attention to eliminate surface contamination from seeds. Furthermore, numerous studies have highlighted the significance of *in vitro* seed sterilization and germination techniques as pivotal tools for addressing the constraints encountered in traditional breeding methods [19,20]. These techniques encompass aseptic germination of seeds in a controlled environment following surface sterilization procedures [7]. Although complete elimination of contamination may not always be feasible, the severity of its repercussions can be mitigated by reducing its frequency through chemical disinfection methods [21].

There are different types of disinfectants used in surface sterilization of plant material. The most commonly used among these are sodium hypochlorite (NaOCl), hydrogen peroxide (H_2O_2), ethanol (C_2H_6O), mercuric chloride ($HgCl_2$), silver nitrate ($AgNO_3$), calcium hypochlorite ($Ca(ClO_2)$), bromine water and Tween 20 [22]. In addition, they may have toxic effects depending on factors such as the applied sterilization method used, concentration of the sterilant, application times or processing sequences [23]. While the sterilization method aims to inactivate all microorganisms, it can also damage plant tissue. For this reason, appropriate combination of treatment the application time and sterilant selection will depend on the sensitivity of the explant to be sterilized. After sterilization, the biological activities of the explant must be preserved [4]. To generalize, the chemicals used in the sterilization of tissue culture materials should be effective, inexpensive, available, and have low toxicity. Therefore, it is important to determine the appropriate sterilant concentration and treatment time to obtain more efficient results, minimize explant damage, and standardize the disinfection method depending on the plant material [17]. In this study, the primary objective was not to completely eliminate all microorganisms from the seed surface (sterilization) but to reduce contamination particularly fungal contamination to obtain healthy and uncontaminated seeds. Accordingly, we aimed to optimize the surface disinfection of bread wheat seeds by determining the most effective treatment conditions in an *in vitro* Murashige and Skoog (MS) medium and petri dish culture. The study evaluated the effects of commonly used disinfectants, including sodium hypochlorite, hydrogen peroxide, and ethanol, by varying their concentrations and treatment durations. This process represents the initial stage of plant cultivation in molecular breeding studies (Figure 1).

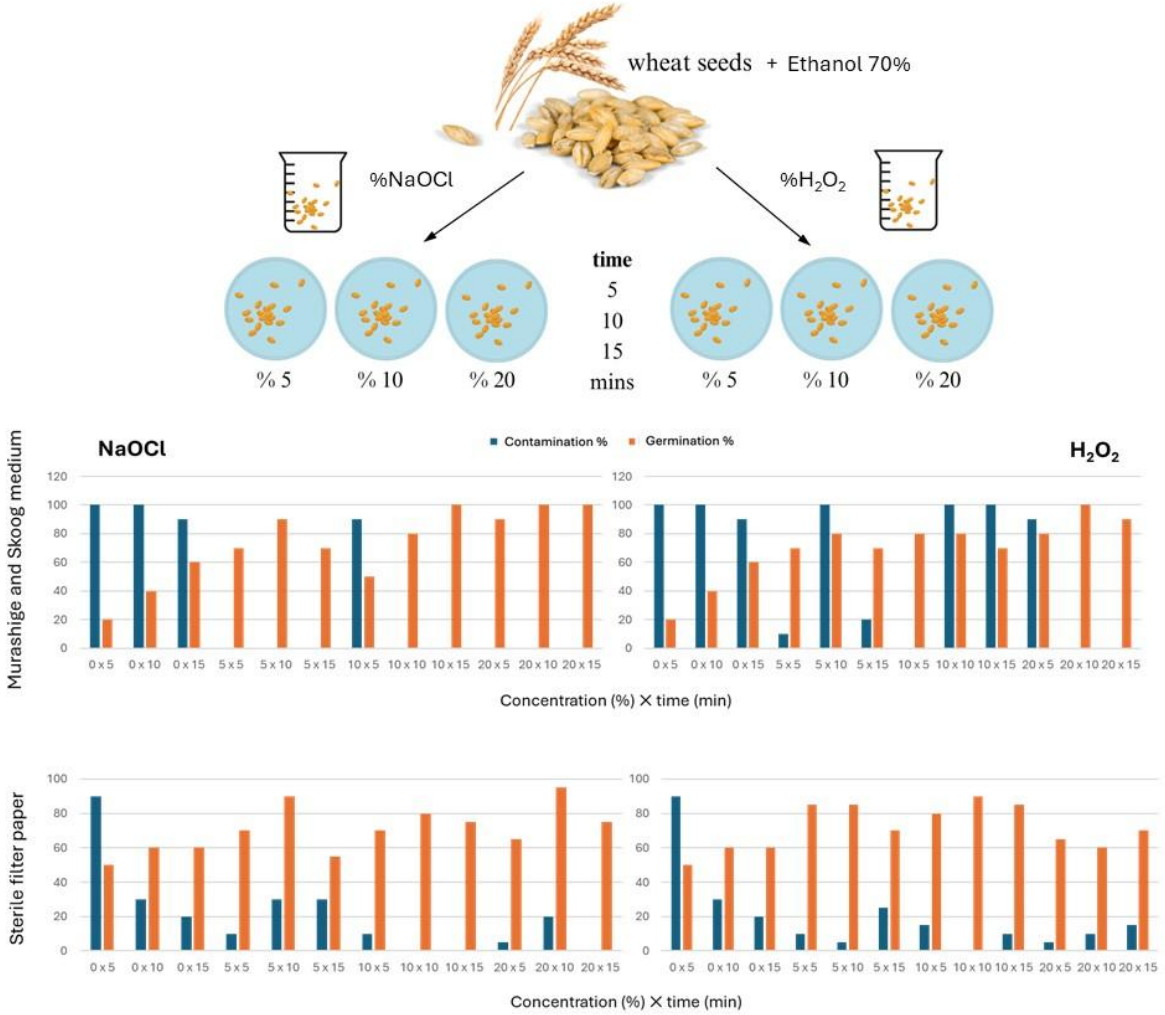

Figure 1. Schematic illustration of optimizing *in vitro* seed disinfection for wheat seeds.

II. MATERIAL AND METHOD

A. Plant Material

In this study, seeds of the bread wheat variety Sultan 95 were used as plant material. If plant seeds are collected from open fields and stored under inappropriate conditions, *in vitro* seed germination is typically inhibited [24] In this study, wheat seeds grown and stored in the field were used, which necessitated the development of a more effective surface disinfection protocol. The seeds were selected for this study due to their natural state and absence of protective fungicidal coating. All experiments were carried out at Karamanoğlu Mehmetbey University, Department of Bioengineering, Molecular Biology and Genetics Laboratory.

B. Surface Disinfection of Wheat Seeds

Commercial domectic bleach (5% NaClO), ethanol (Merck), and hydrogen peroxide (H_2O_2) (Sigma) were used in the study. The disinfectants used were applied at different concentrations and treatment times. In total, 19 different disinfection methods were applied (Table 1). Accordingly, the seeds were washed several times in distilled water and then sterilized in 70% ethanol solution for 1 minute. Then, the seeds were soaked in NaClO and H_2O_2 solutions at different concentrations (0%, 5%, 10%, and 20%) and different treatment (or soaking) times (5, 10, 15 min). Finally, the seeds were washed three times in sterile pure water for 5 minutes each, and each petri dish, 10 seeds were used.

Table 1. Nineteen different surface disinfection methods used for bread wheat seeds.

Methods	Ethanol	NaClO	H_2O_2	Sterilized water
1	70% ethanol – 1 min	5% – 5 min	-	100 ml – 5 min x3
2	70% ethanol – 1 min	5% – 10 min	-	100 ml – 5 min x3
3	70% ethanol – 1 min	5%– 15 min	-	100 ml – 5 min x3
4	70% ethanol – 1 min	10% – 5 min	-	100 ml – 5 min x3
5	70% ethanol – 1 min	10% – 10 min	-	100 ml – 5 min x3
6	70% ethanol – 1 min	10% – 15 min	-	100 ml – 5 min x3
7	70% ethanol – 1 min	20% – 5 min		100 ml – 5 min x3
8	70% ethanol – 1 min	20% – 10 min		100 ml – 5 min x3
9	70% ethanol – 1 min	20% – 15 min		100 ml – 5 min x3
10	70% ethanol – 1 min	-	5% – 5 min	100 ml – 5 min x3
11	70% ethanol – 1 min	-	5% – 10 min	100 ml – 5 min x3
12	70% ethanol – 1 min	-	5%– 15 min	100 ml – 5 min x3
13	70% ethanol – 1 min	-	10% – 5 min	100 ml – 5 min x3
14	70% ethanol – 1 min	-	10% – 10 min	100 ml – 5 min x3
15	70% ethanol – 1 min	-	10% – 15 min	100 ml – 5 min x3
16	70% ethanol – 1 min	-	20% – 5 min	100 ml – 5 min x3
17	70% ethanol – 1 min	-	20% – 10 min	100 ml – 5 min x3
18	70% ethanol – 1 min	-	20% – 15 min	100 ml – 5 min x3
19-control	70% ethanol – 1 min	-	-	100 ml – 5 min x3

C. Germination and culture conditions

After the disinfection process, the seeds were grown in sterile Petri dishes containing 4.4 g/l Murashige and Skoog (MS) [25] supplemented with 30 g/l sucrose (Merck) and 8 g/l Plant Agar (Sigma). For MS, the pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 15 minutes.

Ten wheat seeds were plated in each Petri dish containing autoclaved double-layer sterile filter paper (Whatman filter paper) and the Petri dish containing MS medium. All disinfection procedures were carried out in a laminar airflow sterile cabinet (Nüve LN 090), and all each method included three replicates per treatment.

All Petri dishes containing sterile seeds were left in the dark for two days and then incubated at a temperature of 23±2°C and relative humidity of 60-70% for 16 hours of light and 8 hours of darkness, for 21 days. Wheat seeds started germinating two days after sowing, and germination percentages and contamination rates were recorded after 7 and 21 days.

D. Statistical Analysis

At the end of 21 days, germination and contamination percentages for each petri dish were measured and statistically analyzed. Analysis of variance (ANOVA) test was performed, and Duncan's multiple range test was used for analysis ($p<0.05$) [4,26].

III. RESULTS

In this study, two type surface disinfectant materials (sodium hypochlorite and hydrogen peroxide) were prepared at different concentrations (5, 10, 20%) and treatment times (5, 10, and 15 mins) to maximize the germination of bread wheat seeds. Eighteen different surface disinfection method combinations for wheat seeds in each germination medium were evaluated and described in Table 2.

Table 2. Germination and contamination percentages of bread wheat seeds in different germination media (MS medium, sterile filter paper).

Methods	MS Medium		Sterile filter paper	
	Germination (%) ± (SD)	Contamination (%) ± (SD)	Germination (%) ± (SD)	Contamination (%) ± (SD)
1	70.00±2 ab	0±0 a	68.33±7.63 abc	5.00±5 a
2	90.00±0 cde	0±0 a	86.67±5.77 def	11.67±16.07 a
3	70.00±5 ab	0±0 a	68.33±18.92 abc	13.33±15.27 a
4	56.67±7.64 a	33.33±49.32 abc	68.33±7.63 abc	3.33±5.77 a
5	80.00±5 bcde	0±0 a	90.00±10 f	0±0 a
6	93.33±5.77 de	0±0 a	73.33±7.63 bcde	1.67±5.77 a
7	90.00±5 cde	0±0 a	71.67±7.63 abcd	1.67±2.88 a
8	90.00±10 cde	0±0 a	95.00±5 f	8.33±10.40 a
9	95.00±5 e	0±0 a	73.33±7.63 bcde	1.67±2.88 a
10	68.33±2.88 ab	5.00±5 ab	83.33±7.63 cde	3.33±5.77 a
11	80.00±5 bcde	46.67±50.33 abc	85.00±5 cdef	1.67±2.88 a

12	70.0±5 b	10.00±10 ab	71.67±7.63 abcd	25.00±25 ab
13	75.00±5 bc	3.33±5.77 a	85.00±5 cdef	6.67±7.63 a
14	78.33±7.63 bcd	50.00±50.33 bc	88.33±2.88 ef	0±0 a
15	71.6±7.63 b	56.67±45.09 cd	85.00±±15 cdef	3.33±5.77 a
16	80.00±10 bcde	33.33±49.32 abc	70.00±5 abc	3.33±2.88 a
17	90.0±10 cde	1.67±2.88 a	65.00±5 a	6.67±5.77 a
18	90.00±10 cde	0±0 a	71.67±7.63 abcd	3.33±2.88 a
19-control	36.67±15.27 f	96.67±5.77 d	50.00±10 g	46.67±37.85 b
p-Value	<0.001	<0.001	<0.001	.005

± (SD): Standard Deviation
Different letters within columns indicate significant differences ($p<0.05$)

According to the statistical analysis, it was observed that different concentrations and durations of sodium hypochlorite and hydrogen peroxide cause significant effects on germination and contamination for both MS medium and sterile filter paper (Table 2). The best surface disinfection method for MS medium was determined as to be method 9 (95%), and the contamination percentage for this method is very low (0%). This high germination percentage is followed by method number 6, with 93.33%. When NaOCl and H_2O_2 used as disinfectants in surface disinfection in the MS medium are compared, it can be seen that disinfection methods of 20% NaOCl concentrations (methods 7-9) generally give the best germination and lowest contamination rates (0%). The lowest germination rate observed, 56.67%, belongs to disinfection method 4 containing NaOCl. The lowest contamination rate, 0%, belongs to methods 1, 2, 3, 5, 6, 7, 8, 9 and 18. Accordingly, when the contamination results for the *in vitro* MS medium were examined, it was determined that NaOCl had the lowest contamination rates for all other disinfection methods except method number 4. Although H_2O_2 showed higher germination rates than controls, it was significantly lower when compared to NaOCl in preventing contamination.

When the germination and contamination results observed on sterile filter papers in petri dishes are examined, the highest germination percentage belongs to method number 8 with 95%. Next comes method number 5, with a 90% germination rate. The lowest contamination rate, 0%, belongs to method number 5. Additionally, 0% contamination was observed for method 14. Like the MS medium, the highest overall germination rate belongs to the disinfection method using NaOCl at a concentration of 20% (method 8). The lowest germination rate belongs to method number 17, at 65%. The highest contamination value belongs to method number 12, at 25%. It is seen that the lowest germination and highest contamination values for the sterile petri paper germination environment belong to the H_2O_2 disinfectant. In this case, for both germination media (MS and sterile petri paper), the highest germination rates and the lowest contamination rates were achieved using two concentrations of NaOCl (20% and 10%) and varying treatment durations. Figure 2 shows the effects of bacterial and fungal contamination on different germination media over 21 days of monitoring in Petri dishes.

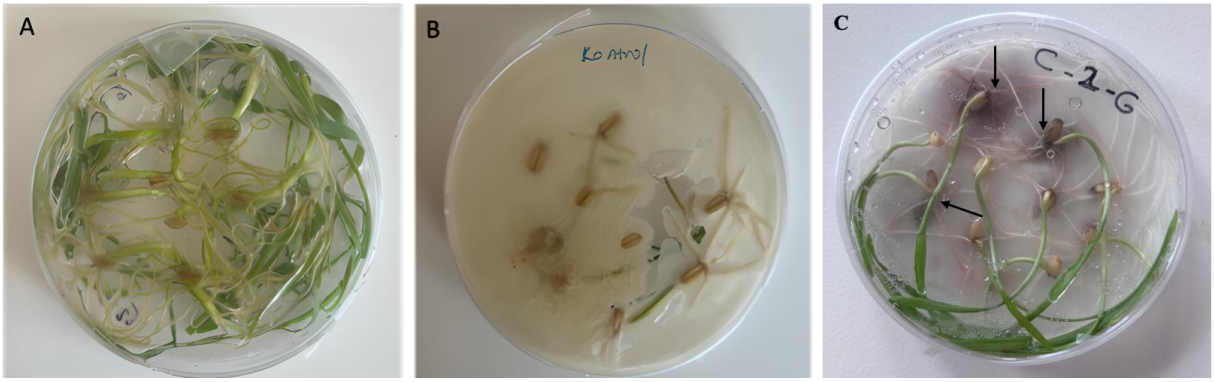

Figure 2. Effects of contamination on germination over 21 days, showing various levels of germination and contamination A) germinating healthy wheat seeds in MS medium B) wheat seeds showing contamination (bacterial) in *in vitro* MS medium (control group) without any disinfection C) wheat seeds contaminated by fungal contamination on sterile petri paper, with contamination indicated by arrows.

When germination percentages are examined in relation to disinfectant treatment durations, it is observed that the highest germination rates were obtained with methods 6, 8, 9, and 17, which involved 10- and 15-minute disinfection times.The lowest germination percentage belongs to method number 4 with a treatment time of 5 minutes. For NaOCl, the germination percentage increased even as the treatment time increased, and for H_2O_2, although the increase treatment time was higher for 10 min than for 5 min, it was lower for 15 min than for 10 min. When the contamination percentages are examined in terms of treatment duration with disinfectants, it is seen

that 5 minutes of treatment causes more contamination in petri dishes than 10 and 15 minutes. It was observed that 10 and 15-minute treatment times did not suppress contamination being observed in the petri dishes at all concentrations applied for NaOCl.

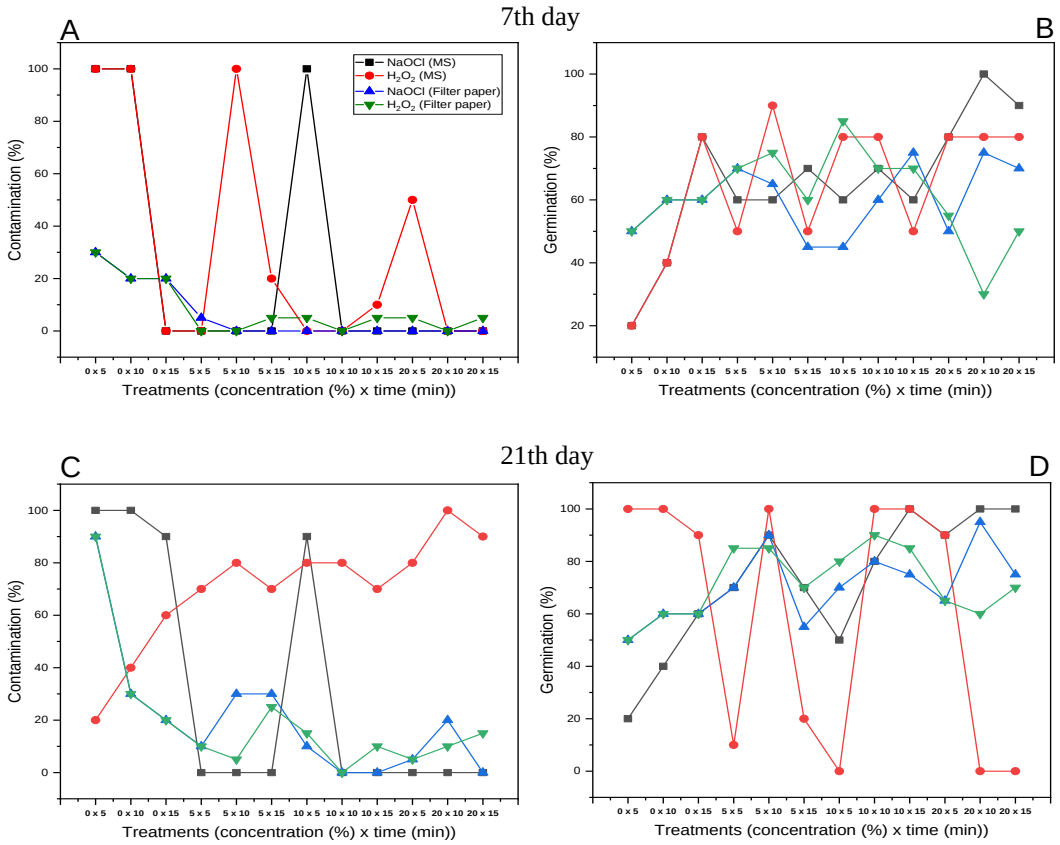

Figure 3. Effects of NaOCl and H_2O_2 on contamination and germination rates at the 7th and 21st days in different germination media (MS and Sterile Filter Paper). (A) Contamination rates at the 7th day, (B) Germination rates at the 7th day, (C) Contamination rates at the 21st day, and (D) Germination rates at the 21st day.

The effects of germination and contamination in MS medium and sterile filter paper environments were comparatively examined on the 7th and 21st days of germination (Figure 3). By the 7th day, it was observed that NaOCl usage minimized contamination in all treatments except for one specific concentration trial (%10 x 5 min) (Figure 3A). On the 21st day, as expected, control samples treated only with ethanol (common to all seeds) and rinsed with sterile water for varying durations exhibited high contamination rates in the absence of disinfectant application (Figure 3C). In terms of germination rates, NaOCl treatment demonstrated average effects at both observation points (Figures 3B and 3D). At higher concentrations (20%), NaOCl was highly effective in reducing contamination and promoting germination. These findings indicate that NaOCl, at higher concentrations, protects wheat seeds from contamination without creating any adverse effects on germination. On the other hand, at the end of the 21st day, H_2O_2 negatively affected germination as the application duration and concentration increased. This suggests that higher concentrations of H_2O_2 may exert toxic effects on seeds during prolonged germination periods. It should be noted that prolonged exposure and increased concentrations of disinfectants may harm the seeds, highlighting the necessity of a rinsing and purification step with sterile water following disinfectant treatments, as was conducted in this study (Table 1).

In the MS medium, the contamination rates for H_2O_2 were more inconsistent and higher compared to NaOCl, both on the 7th day and at the end of the 21st day. By the 21st day, H_2O_2 application, particularly at higher concentrations, was found to be highly ineffective in surface sterilization of seeds (Figure 3C). Similarly, in terms of germination rates, H_2O_2 application in the MS medium resulted in irregular and significantly lower germination rates, particularly on the 21st day, mirroring the trends observed in contamination (Figure 3D).

In applications on sterile filter papers, contamination rates for both disinfectants were quite low by the 7th day (Figure 3A), while a slight increase in these rates was observed by the 21st day (Figure 3C), which is an

expected outcome. Measurements of contamination increases at the end of the 21st day indicate the decreasing effectiveness of the disinfectants used over time. When germination rates were compared, the disinfectants exhibited a germination profile similar to that observed in the MS medium, with average germination rates (Figures 3B and 3D). In a study observing seed germination in potting soil and on sterile filter paper, the results highlighted certain advantages of the sterile filter paper in terms of the sterilization environment. Specifically, the sterile filter paper provided a controllable environment for monitoring contamination, as it allowed for easy tracking of differences such as fungal contamination and color changes [27]. In a study involving *Hibiscus coddii* subsp. *barnardii* seeds, the effects of the culture medium on seed germination were tested across various germination environments, including MS medium and filter paper [28]. According to the study results, the low-strength MS medium and sterile filter paper environment exhibited high germination rates. In another study, it was reported that sterile filter paper demonstrated the highest germination rates (92.05%) compared to a pot environment containing sand and compos [29]. These results highlight the potential of sterile filter paper as a cost-effective and practical alternative to the MS medium, particularly for surface sterilization and germination efficiency. Compared to the MS medium, the lower nutrient content and reduced moisture in the sterile filter paper environment create unfavorable conditions for microbial growth.

IV. DISCUSSION

In this study's initial disinfection (pre-treatment) phase, all wheat seeds, including the control groups, were subjected to a one-minute treatment with 70% ethanol. Despite ethanol's efficacy as a sterilizing agent, it is crucial to acknowledge its potential phytotoxic effects. Furthermore, previous studies have indicated that a two-stage sterilization process involving ethanol can be more effective [22]. In the context of this study, wheat seeds in petri dishes (19), serving as controls with only ethanol application, exhibited both lower germination rates and higher contamination percentages compared to methods where both disinfectants were utilized.

Sodium hypochlorite is frequently used for sterilization of explant sources *in vitro* culture studies, as it is simple, inexpensive, and readily available, and is effective against all kinds of contaminants such as bacteria, fungi, and viruses [30–32]. NaOCl is also used in molecular breeding studies during the surface disinfection phase of wheat seeds germinated on sterile filter papers [16,33,34]. It is also employed in studies combining wheat with various tissue culture experiments within classical breeding programs [35].

Örgeç et al. [4] reported that among the different disinfection methods they applied for einkorn and einkorn-like grains, the best germination rates belonged to methods 2 and 12 and 1-min method with 70% ethanol followed by 10 and 15 min methods of 20% NaOCl. The results we obtained in this study support the results obtained by Örgeç et al. [4]. Hesami et al. [17] conducted a study to assess the seed germination and contamination of Ficus religiosa, in which six different concentrations of sodium hypochlorite (0%, 5%, 10%, 15%, 20%, and 25%) and three different treatment (soaking) durations (5, 10, and 15 minutes) were examined. The lowest contamination rate (0%) for seeds germinating in MS medium was 10- and 15-min treatment times in sodium hypochlorite at 20% concentrations. The highest seed germination rate was observed for 5- and 10-minute treatment with 10% concentration. A 20% NaOCl concentration also indicates the lowest contamination rate in this study, and in this respect, a result that overlaps with the literature has emerged. However, the study by Hesami et al. [17] reported that high concentrations of NaOCl caused a blackish color on the seeds and a low germination rate. In contrast, our study revealed that high NaOCl doses (20%) showed high germination percentages for wheat seeds, and did not cause any damage to the seeds. Hasemi et al. [19] stated that 20% NaOCl concentration is the best germination rate for einkorn-like grains. In this regard, it should be taken into consideration that increasing NaOCl concentrations may have a negative effect on the plant depending on the sensitivity of the plant used as an explant.

In a study aimed at developing an effective surface disinfection protocol to overcome conditions inhibiting the *in vitro* germination of cotton seeds, various disinfecting agents such as bleach, chlorine gas, and hydrogen peroxide, as well as additional treatments like soap-water washing and ethanol rinsing, were tested. The findings revealed that hydrogen peroxide treatment significantly improved germination rates and reduced contamination compared to other methods [24]. According to a study by Dolatabadian & Modarressanavy [36] hydrogen peroxide was reported to be more effective than other sterilization factors for plant tissue. However, different studies also show that hydrogen peroxide is ineffective in surface disinfection of explants and seeds [37]. Al Ghasheem et al. [38] tested four disinfection agents (NaOCl, H_2O_2, Captan fungicide, and boric acid) in 8 different variants for *in vitro* cultures of peach explants. According to the study's results, it was determined that sodium hypochlorite showed the best germination performance, but the hydrogen peroxide results were not found satisfactory enough. In our research, NaOCl was found to have better germination and lowest contamination performance than H_2O_2 for wheat seeds.

It has been reported that seeds collected from open fields or stored under improper conditions are generally severely infected by various fungi compared to seeds stored in a controlled environment [24]. In Figures

2B and 2C, bacterial and fungal contaminations were observed as expected contamination types in the seeds examined (Figure 2). The occurrence of such contamination is largely attributed to the use of wheat seeds that were field-grown, stored, and in their natural state without protective fungicide coatings. Therefore, it is clear that when seed supply is limited, particularly with seeds heavily contaminated, *in vitro* germination presents a significant challenge.

This study aims to determine the most effective disinfection conditions for wheat germination by examining different disinfectant types, concentrations, and application times. Therefore, in this study, the effects of two commonly used disinfectant types, sodium hypochlorite and hydrogen peroxide, on *in vitro* seed disinfection in the MS medium for wheat germination were evaluated in terms of various concentrations (5%, 10%, 20%) and treatment times (5, 10, 15 minutes). Additionally, as a first step for molecular and breeding studies in wheat, the effects of surface disinfection on germination and contamination rates in petri dishes containing sterile filter papers were also examined. As a result of the evaluations made among a total of 19 different disinfection methods, the most effective disinfection conditions for the *in vitro* MS environment were obtained at 20% concentration of NaOCl and treatment for 15 minutes. The germination rate obtained under these conditions was determined as 95%, and the contamination percentage was also determined as 0%. Based on the germination and contamination results observed in petri dishes containing sterile filter paper, the highest germination rate (95%) was comparison to H_2O_2, NaOCl demonstrates effective surface disinfection in wheat germination across various germination media. The results of the study contribute to determining the optimal disinfectant conditions recommended for surface disinfection in wheat germination, providing insights for both *in vitro* seed germination studies and molecular breeding research.

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