The Effect of Several Sterilization Techniques in String Bean Tissue Culture

Irmawati*, Firdaus Sulaiman, Zaidan P. Negara, Marlina, Lidwina N. Sulistyaningsih, Dinda Asari, Laras Indah Lestari
{irmawati@fp.unsri.ac.id}

Department of Agronomy, Faculty of Agriculture, Universitas Sriwijaya.

Abstract. Tissue culture is a plant propagation technique in which plant parts such as cells, tissues, organs or the whole plant are isolated under aseptic conditions. The aim of this study is to find the best sterilization technique for tissue culture of string beans. This study was conducted in tissue culture laboratory of Faculty of Agriculture, Universitas Sriwijaya. The treatments consisted of 6 sterilization methods, each of which consisted of a combination of different sterilizing agents (fungicide, detergent, bleach solution, and alcohol) and the duration of sterilization. Based on the results, it was found that treatment S4 with a 0.2% fungicide for 15 minutes, followed by a detergent for 3 minutes, a 20% bleach solution (with a sodium hypochlorite active ingredient of 5.25%) for 20 minutes, and 70% alcohol for 10 minutes provided the best results, as evidenced by a relatively lower percentage of contamination and a higher percentage of live explants compared to the other treatments.

Keyword: Effect, Sterilization Techniques, String Bean, Tissue Culture

1. Introduction

Tissue culture or in vitro culture is a plant propagation technique in which plant parts such as cells, tissues, organs or whole plants are isolated under aseptic conditions by providing nutrients and controlled environmental conditions [1]. The advantages of tissue culture propagation technique are the production of a large number of plants in a relatively short time [2], they are high quality, uniform, free from pests and pathogens [3], [4] and can be continuously available. Tissue culture techniques are widely used to produce high quality seed for annual plants [5]–[7]. The use of tissue culture for string bean is mainly to produce high quality and virus-free varieties. The string bean itself is one of the most important vegetable crops in Indonesia as it is used in a variety of traditional dishes.

One of the most limiting factors for tissue culture is the increased risk of contamination after explant inoculation by fungi or bacteria [8], [9]. Therefore, the sterilization procedure plays an important role in the success of tissue culture. The purpose of explant sterilization is to prevent or kill microorganisms that might be transferred or attached during explant isolation [10]. Various sterilization methods have been used that are expected to effectively eliminate the source of contaminants present in explants [11], [12].

The combination of sterilant and appropriate immersion time is a critical factor in the success of sterilization [13]. There are many types of sterilants that can be used for explant sterilization, such as sodium hypochlorite (NaClO), mercuric chlorite, detergents, and 70%

SRICOENV 2022, October 05-06, Palembang, Indonesia

DOI 10.4108/eai.5-10-2022.2328348

alcohol. In a study [14], it was reported that the use of 70% alcohol for 5 min and 10% sodium hypochlorite solution for 10 min was the best treatment for sterilization of oil palm explants. Thus, this study is an approach to provide information on the proper sterilization method to minimize contamination in tissue culture of string bean plants.

2. Materials and Method

This research was conducted in the Tissue Culture Laboratory, Department of Agronomy, Universitas Sriwijaya, Palembang Campus with coordinates 2°59'23.4 "S 104°43'53.4 "E. The research phases included MS media preparation, isolation and sterilization of explants, inoculation of explants into media, and observation. Observations were used to monitor the growth and development of the explants. Parameters observed included percentage of contamination (%), percentage of live explants (%), percentage of bud sprouting (%), and any visual changes on the explants.

The treatments consisted of six sterilization procedures combining different sterilizing materials and the duration of each sterilization procedure. All six treatments are listed in Table 1 below. The string bean seeds were first germinated in a sterilized vessel for several days before being isolated and subjected to the sterilization procedure. The explants consisted of the seeds and different parts of string bean seedlings, such as hypocotyl, plumule and radicle.

Table 1. Six sterilization treatments on string bean tissue culture

Treatment	Sterilant materials and sterilization duration
S1	Detergent for 3 minutes + 20% bleach solution for 20 minutes + 70% alcohol for 10 minutes
S2	Detergent for 3 minutes + 20% bleach solution for 15 minutes + 70% alcohol for 10 minutes
S3	Detergent for 3 minutes + 20% bleach solution for 10 minutes + 70% alcohol for 10 minutes
S4	0.2% Fungicide for 15 minutes + detergent for 3 minutes + 20% bleach solution for 20 minutes + 70% alcohol for 10 minutes
S5	0.2% Fungicide for 15 minutes + detergent for 3 minutes + 20% bleach solution for 15 minutes + 70% alcohol for 10 minutes
S6	0.2% Fungicide for 15 minutes + detergent for 3 minutes + 20% bleach solution for 10 minutes + 70% alcohol for 10 minutes

The sterilants used were Mama Lime dish washer detergent, Bayclin (with a sodium hypochlorite active ingredient of 5.25%), and Benomyl fungicide.

3. Results and Discussion

Observation of explants was performed immediately after inoculation. Inoculated explants were placed in jars containing Murashige and Skoog (MS) culture media and then stored in a room with controlled conditions. As shown in Fig. 1, contamination was highest in the first week after inoculation, with the exception of S3, where the highest percentage of contamination occurred in the second week after inoculation. The explants of S5 treatment had the highest level of contamination at 65%, while S4 had the lowest level of contamination at only 25% in the first week after inoculation. Contamination decreased after 7 days, as shown

in Fig. 1, where all percentages were much lower thereafter, except for S3, where the contamination percentage peaked at 50% 14 days after inoculation.

The contaminations that occurred were due to infection with fungi or bacteria, as evident from the explants and culture media. The most noticeable changes were the formation of fungal hyphae colonies on the surface of culture media and explants. This is a common contamination problem in tissue culture that has been investigated in many study reports [8], [15]. Bacterial contamination would cause the plant to become wet or produce mucus as the bacteria directly attack the plant tissue [16].

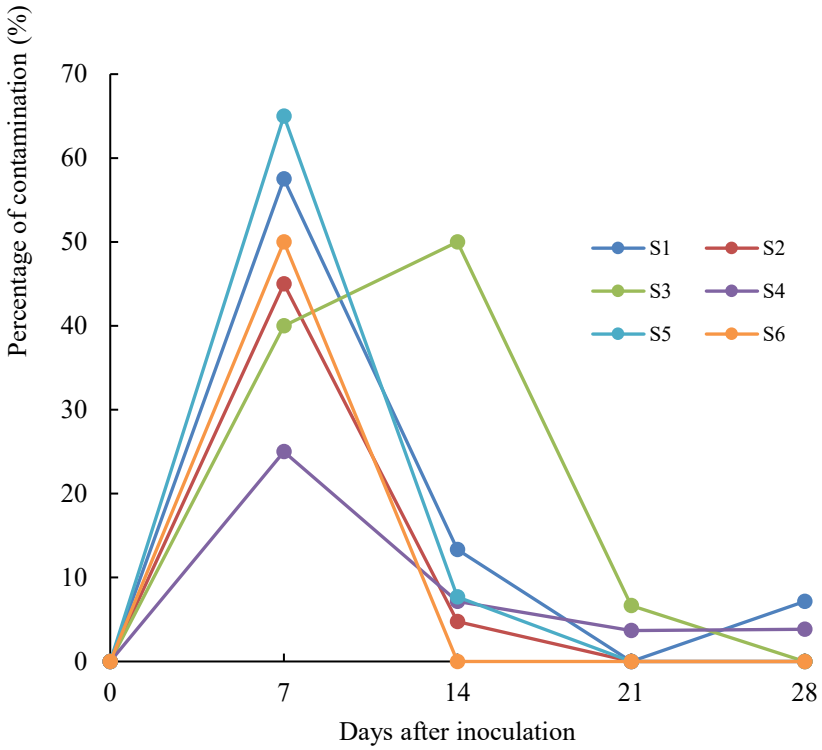
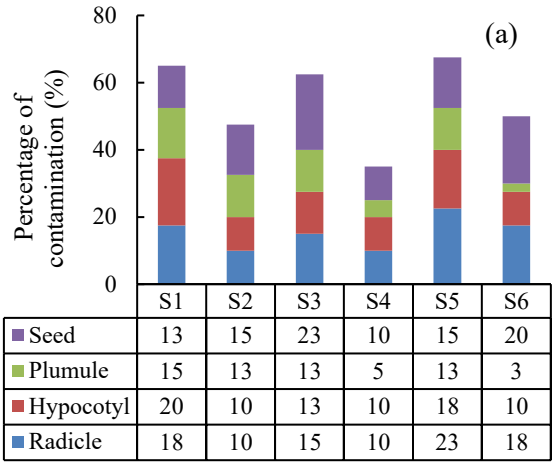
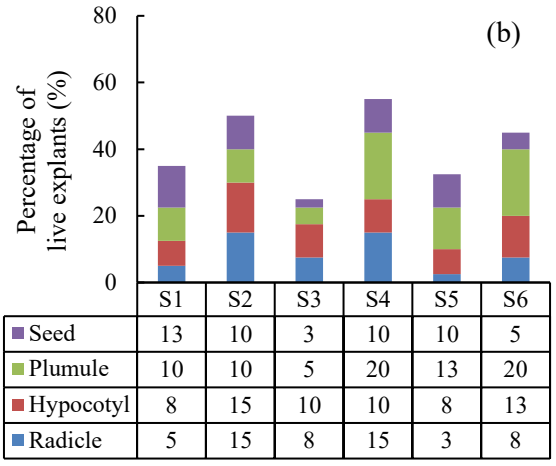


Figure 1. Weekly percentage of contamination due to the sterilization treatments on string bean explants

Figure. 1 shows the percentage of contamination and the percentage of live explants out of the total number of inoculated explants at the fourth week after inoculation. The S4 treatment has the lowest percentage of contamination at 35%, and the S5 treatment has the highest at 69%. Fig. 2a also shows that contamination was mainly from explants derived from seeds and radicles. Contamination from seeds was highest in the S3 treatment, where it accounted for 23% of the total 64% contamination. While contamination from radicle-derived explants accounted for approximately 23% of the total contamination in the S5 treatment out of a total of 69%. This result is in contrast to the research results of [17], where explants isolated from seeds and roots had the lowest contamination rate.



	S1	S2	S3	S4	S5	S6
Seed	13	15	23	10	15	20
Plumule	15	13	13	5	13	3
Hypocotyl	20	10	13	10	18	10
Radicle	18	10	15	10	23	18

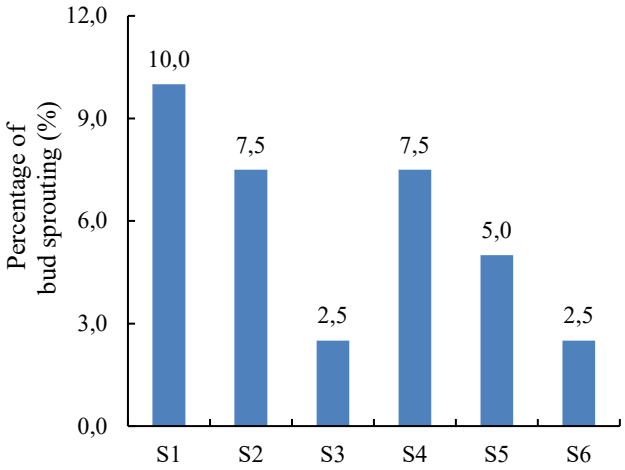


	S1	S2	S3	S4	S5	S6
Seed	13	10	3	10	10	5
Plumule	10	10	5	20	13	20
Hypocotyl	8	15	10	10	8	13
Radicle	5	15	8	15	3	8

Figure 2. The percentage of contamination (a) and percentage of live explants (b) from total inoculated explants at the 4th week after inoculation.

The percentage of live explants was calculated from all remaining, uncontaminated explants, regardless of whether callus development, bud burst, or even no changes were observed on the explants as long as no contamination was present, as shown in Fig. 2b. From the results, S4 was the treatment with the highest percentage of live plants at 55%. This also corresponded to the percentage of contamination, with S4 being the treatment with the lowest contamination. The explants isolated from the plumules contributed the most to the percentage of live explants, with the highest percentage being both 20% of a total of 55% in S4 and of a total of 46% in S6.

The percentage of bud burst was quite low in all treatments, as can be seen in Fig. 3 below. The highest bud formation was observed in treatment S1 with only 10%, which means that only 4 explants formed buds out of a total of 40 explants. The lowest percentage was only 2.5% in treatments S3 and S6, which means that only one explant formed buds out of a total of 40 explants. It was also observed that most of the buds emerged from seed explants, except for S5 where buds also emerged from hypocotyl explants.



Figyre 3. Percentage of bud sprouting in string bean explants at the 4th week after inoculation

The timing of bud break varied among treatments. Bud burst from seed-isolated explants occurred in the first week after inoculation in all treatments except S6, where bud burst began three weeks after inoculation. Two weeks after inoculation, bud break still occurred in treatments S2 and S4. Four weeks after inoculation, S2 and S6 had 0% contamination of bud-emerging explants, while S1 had 33% contamination and S3, S4, and S5 had 50% contamination (data not shown).

4. Conclusions

From the results of the study, it was concluded that sterilization with a combination of 0.2% fungicide for 15 minutes, followed by detergent for 3 minutes, then 20% bleach solution for 20 minutes, and finally 70% alcohol for 10 minutes (S4 treatment) was the best sterilization method for string bean tissue culture because it had the lowest percentage of contamination and the highest percentage of live explants.

Acknowledgement

The research of this article was funded by DIPA of Public Service Agency of Universitas Sriwijaya 2022. SP DIPA-023.17.2.677515/2022, on December 13, 2021. In accordance with the Rector's Decree Number: 0109/UN9.3.1/SK/2022, on April28, 2022.

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