Agrotechnology Research Journal
Volume 8, No. 1, June 2023, pp. 37–42

ISSN 2655-7924 (Print)
ISSN 2614-7416 (Online)
https://jurnal.uns.ac.id/arj
doi:10.20961/agrotechresj.v8i1.90917

Bacteria Elimination of *Xanthomonas axonopodis* pv. *glycine* and Improvement of Viability of Soybean Seeds Through a Combination of Temperature and Duration of Dry Heat Treatment

Aprilia Diandra Savitri, Imelda Milaros, Irene Patria Hannyvone, Evan Purnama Ramdan*

Department of Agrotechnology, Faculty of Industrial Technology, University of Gunadarma, Depok, 16424 West Java, Indonesia

Received **February 27, 2024;** Accepted **May 22, 2024**

ABSTRACT

Soybean is the main food crop in Indonesia, besides rice and corn, and its need continues to increase. *X. axonopodis* pv*. glycine* (Xag) is a pathogen that causes bacterial pustule disease in soybeans, which can cause soybean productivity to decrease by 57.61%. Xag is also a seed-borne pathogen that spreads through seeds, reaching 8%. Therefore, this study aims to determine the optimum combination of dry heat treatment techniques to eliminate Xag and increase seed viability and vigor. The study was arranged in a randomized complete factorial design consisting of 2 factors: the temperature factor of control, 25 and 45°C, and the duration factor of 6, 12, and 18 hours. Soybean seeds inoculated with Xag were then given a dry heat treatment (DHT) treatment of 100 seeds each. Then, the population of Xag bacteria, germination, vigor index, and seed viability were calculated. The results showed that DHT with a temperature of 45 °C was the best in suppressing the Xag population but reduced soybean seed viability. Therefore, the optimal combination of DHT to eliminate and maintain seed viability is at 25 °C for 12 hours.

Keywords: Bacterial pustule; Germination rate; *Glycine max;* seed borne; Vigor index

***Cite this as (CSE Style)*:** Savitri AD, Milaros I, Hannyvone IP, Ramdan EP. 2024. Bacteria elimination of *Xanthomonas axonopodis* pv. *glycine* and improvement of viability of soybean seeds through a combination of temperature and duration of dry heat treatment. Agrotechnology Res J. 8(1):37–42. https://dx.doi.org/10.20961/agrotechresj.v8i1.90917.

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is the main food crop in Indonesia, in addition to rice and corn. Soybean is one of the sources of vegetable protein that can be utilized in raw materials for food industries, such as milk, tempeh, tofu, soy sauce, and others (Krisnawati 2017). Direct consumption of soybeans in Indonesia in 2021 reached 2.8 million tons, and more than 500 thousand tons were used as processed products such as flour, vegetable oil, soy sauce, and soy milk. Domestic soybean production (30% of the national budget) still needs to meet the national consumption of soybeans, so the rest must be met through soybean imports (Indonesian Ministry of Agriculture 2020). In 2020, there was an increase in soybean production in Indonesia from 14.87 quintals per hectare to 14.94 quintals per hectare. However, the total production still needs to meet the national production target of 16.58 quintals per hectare (Indonesian Central Bureau of Statistics 2021).

The challenge in increasing soybean production is using superior seeds that have high viability and vigor and are free from seed-borne pathogens. Fauziyah et al. (2022) have successfully detected *Xanthomonas axonopodis* pv. *glycines* (Xag) bacteria in soybean seeds with populations reaching 5.2×10^3 to 7.5×10^3 CFU mL^{-1}. The presence of seed-borne pathogens can cause a decrease in seed viability and vigor, death in the nursery, and disease explosion in the field (Amza 2018; Ramdan et al. 2020; Martín et al. 2022). Meanwhile, *X. axonopodis* can cause a decrease in soybean productivity of up to 57.61% (Rumbiak et al. 2018) and the spread of bacteria through seeds can reach 8% (Habazar et al. 2012). This infection affects the harvest and can cause significant economic losses in the agricultural sector. Therefore, it is essential to keep seeds free from pathogens so that soybean productivity remains optimal.

One method that can potentially suppress the seed-borne pathogen infection rate is the dry heat treatment technique (Ramdan et al. 2020). Dry heat is a common physical method for the safe treatment of various types of seeds that can effectively eradicate pathogens from seeds (Shi et al. 2016; Godefroid et al. 2017; Soni et al. 2022). Dry heat treatment is also an environmentally friendly alternative for reducing pathogen infections in seed (Falconí and Yánez–Mendizábal 2016). Fauziyah et al. (2024) found that the dry heat treatment technique at a temperature of 45 °C for 24 hours on soybean seeds successfully eliminated *X. axonopodis* bacteria. Dry heat treatment can also affect seed viability and vigor (Farooq

****Corresponding Author*:**
E-Mail: evan_ramdan@staff.gunadarma.ac.id

et al. 2008; Samarah et al. 2021). Therefore, it is necessary to combine variations in temperature and duration of treatment that effectively eliminate *X. axonopodis* bacteria and increase seed viability and vigor so that it is expected to provide new contributions in managing soybean seed diseases more effectively and sustainably. This study aimed to determine the optimal combination of dry heat treatment techniques to eliminate Xag and the optimal combination of dry heat treatment techniques to increase seed viability and vigor.

MATERIALS AND METHODS

Time and location

The research was conducted from May to August 2024. The research location was at the Intermediate Agrotechnology Laboratory, Agrotechnology Study Program, Faculty of Industrial Technology, Gunadarma University, Campus F7, Ciracas, East Jakarta.

Research methods

This research was designed using a Completely Randomized Factorial Design consisting of 9 combinations of temperature factor consisting of 3 levels (0, 25, and 45 °C) and duration factor consisting of 3 levels (6, 12, and 18 hours). Then, each treatment was repeated three times.

Research procedure. 1) Preparation of Xag bacterial isolates, Xag bacterial isolates were prepared by growing isolates on petri dishes containing Tryptic soy agar (TSA) media; 2) Preparation of soybean seeds, the soybean seeds to be used are soybean variety Dering three obtained from the Seed Source Management Unit (UPBS) of Balitkabi. Seeds are sorted first from dirt and damaged seeds; 3) Inoculation of soybean seeds with Xag bacteria, Xag bacteria are propagated by adding one colony of bacteria to 1 L of Tryptic soy broth (TSB) media. It was then shaken for 24 hours on an orbital shaker. Then, the cell density of the bacterial suspension was calculated and prepared to reach 103 CFU.mL^{-1}. After the bacterial suspension was ready, 350 soybean seeds were soaked in the Xag bacterial suspension for 30 minutes and then dried (Nikko et al. 2023); 4) Application of dry heat treatment on soybean seeds, Seeds that have been inoculated with Xag bacteria are then heated in a dry oven according to the combination of temperature and duration that has been determined as in Table 1. In each treatment used 100 seeds.

Variable observations. First, the population of Xag bacteria was calculated using the liquid assay method (Fauziyah et al. 2022). Seeds given dry heat treatment were weighed, and 110 mL of sterile distilled water was mashed. The soybean seed suspension was then serially diluted to 10^{-4}. Each 0.1 mL of soybean suspension from dilutions 10^{-3} and 10^{-4} was cultured on TSA media by scatter and incubated for two days. Calculation of bacterial population using the formula (Nikko et al. 2023) as follows [1].

$$N = \frac{\sum c}{[1\times n_1]+[0{,}1\times n_2]\times[d]} \quad \text{.....[1]}$$

with *N*= number of colonies (CFU mL^{-1}), $\sum$c= number of colonies in all counted Petri's dishes, n_1= number of cups in the first dilution, n_2= number of Petri's dishes in the second dilution, and *d*= first dilution. Second, soybean seed viability and vigor test, A total of 25 soybean seeds from each treatment combination were grown using the blotter paper method with three replications. Calculation of germination and seed viability was observed on the 5th and 14th days after sowing. Seeds were observed for normal and abnormal sprouts on the seventh day after sowing. Calculation of seed germination (SG) and viability (SV) were calculated using the formula (ISTA 2010) as follows [2, 3].

$$SG = \frac{\Sigma\, KN\ I + \Sigma\, KN\ II}{\Sigma\, seeds\ planted} \times 100\% \text{.....[2]}$$

with *SG*= germinated seeds, *Σ KN* I= normal sprouts of the first observation (5 DAP), and *Σ KN II*= regular sprouts of the second observation (14 DAP).

$$SV = \frac{\Sigma\, sprouts\ that\ grew\ on\ the\ 14th\ day}{\Sigma\, normal\ sprouts\ on\ the\ 1st\ observation} \times 100\% \text{....[3]}$$

with SV= viable seeds.

Meanwhile, the vigor index (VI) was calculated using the formula (ISTA 2010) as follows [4].

$$VI = \frac{\Sigma\, normal\ sprouts\ on\ the\ 1st\ obsertation}{\Sigma\, seeds\ planted} \times 100\% \text{....[4]}$$

The data was obtained and then tested statistically using analysis of variance with SAS 9.1 software. The data showing a significant difference continued with Tukey's further test at the 5% level.

RESULTS AND DISCUSSION

Population suppression of *Xanthomonas axonopodis* pv. *glycine* on soybean seeds

Variance analysis showed a significant effect of dry heat treatment (DHT) duration and temperature (Table 1). The duration of DHT treatment for 18 hours showed a significant effect compared to the duration of 6 hours. The duration of DHT treatment also showed a pattern that the longer the duration of DHT on soybean seeds, the lower the Xag population (Table 1). Therefore, a longer time will more effectively reduce the bacterial population. As reported by Fauziyah et al. (2024), the longer the duration of heating is, the more suppressed the bacterial population will be. Yousof and Ibrahim (2013), also reported that heating seed for 2 days is a good means for controlling seed-borne disease and enhancement of seed vigor. This finding is significant in the field of plant pathology and agricultural sciences as it suggests that longer DHT treatments can be more effective in reducing the Xag population. Meanwhile, the temperature of the DHT treatment did not show a significant effect. However, based on the combination of temperature and DHT duration, the best treatment to suppress the Xag population is the DHT treatment with a temperature of 25 °C for 18 hours. The treatment was able to suppress the Xag population from 3.28×10^3 CFU L^{-1} to 0.25×10^3 CFU L^{-1} (Table 1).

Table 1. Effect of temperature and duration of Dry Heat Treatment on the population of Xag bacteria in soybean seeds (×10^3 CFU L^{-1})

Dry Heat Treatment (°C)	Duration (hours)			Averages
	6	12	18	
0	3.28±0.35Aa	2.44±0.12ABa	1.74±0.12Ba	2.48±0.24a
25	2.63±0.36Aab	2.19±0.51ABab	0.35±0.47Bab	1.72±0.45ab
45	1.58±0.19Ab	0.78±0.08ABb	0.77±0.40Bb	1.04±.,22b
Averages	2.49±0.29A	1.80±0.24AB	0.95±0.33B	

Remark: Numbers with different capital letters in the same row indicate significant differences in DHT treatment duration (p<0.05). Numbers with different lowercase letters in the same column indicate significant differences from the DHT temperature treatment (p<0.05).

This finding indicates that the DHT treatment was able to suppress the Xag population but could not eliminate 100% of the Xag population. Kurniasih et al. (2020) reported that seed-borne pathogens cannot be eliminated by dry heat treatment alone. Therefore, the control of seed-borne pathogens cannot be completed by one technique alone, so integrated control needs to be carried out as a preventive and curative measure.

Response of germination, seed viability, and vigor index

The treatment of soybean seeds with the DHT technique showed a significant effect on germination, seed viability, and vigor index based on analysis of variance (Table 2) with a value of p< 0.05. The germination test results showed that the DHT temperature factor treatment and the interaction between temperature and DHT duration significantly reduced germination (Table 2). DHT treatment, both temperature factors, duration, and their interactions significantly affected seed viability. In the vigor index response, the natural effect was shown by the temperature factor and the interaction between temperature and DHT duration (Table 2). The vigor index compares the number of usual sprouts in the initial observation and the total seeds planted. The percentage of usual sprouts in the initial observation strongly correlates with the seeds' ability to germinate in the field compared to the percentage of sprouts at the end of the observation (Febriani and Widajati 2015). DHT treatment at 45 °C significantly reduced soybean germination (Table 3). This treatment is also in line with seed viability and vigor index testing. DHT treatment at 45 °C significantly reduces seed viability (Table 4) and vigor index (Table 5). DHT treatment at 25 °C consistently maintained germination, seed viability, and vigor index, as indicated by the lack of significant effect compared to the control. The percentages of germination, seed viability, and vigor index in the 25 °C DHT treatment were 46.44±0.13, 23.11±2.68, and 69.78±0.36%, respectively (Table 3, Table 4, Table 5). Meanwhile, the duration factor only significantly affected the seed viability in the 18-hour DHT treatment compared to the 16-hour DHT (Table 4).

This study also found that the interaction of a DHT temperature of 45 °C with a duration of 6, 12, and 18 hours significantly reduced germination, seed viability, and vigor index (Table 6). Too high temperatures can damage seed cells and reduce vigor; this is supported by the statement of Syahputra and Hadi (2012) that soybean seeds heated at temperatures above 60 ℃ can damage the protein and vitamin content in the seeds due to the process of heat shock proteins (HSPs). Sharma et al. (2022), seedling vigor showed a reduction at higher temperatures. Seed deterioration will probably accelerate with the increase in high temperature (Mansour 2023). Damaged seed membranes can cause a decrease in seed vigor due to the release of electrolyte solutions (Rofiq et al. 2013; Xing et al. 2023).

Table 2. Recapitulation of variance analysis of the effect of temperature, duration, and their interaction on seed germination, viability, and vigor index

Dry Heat Treatment	Germination	Seed viability	Vigor index
Temperature (T)	24.13*	88.64*	29.23*
Duration (D)	0.75ns	4.78*	1.55ns
Interaction of TxD	3.64*	86.57*	5.71*

Remark: *= significant effect at 5% level; ns= no significant effect based on F test

Table 3. Effect of temperature and duration of DHT on germination (%)

Dry Heat Treatment (°C)	Duration (hours)			Average
	6	12	18	
0	35.33±0.08Aa	38.67±0.13Aa	48.00±0.14Aa	40.67±0.17a
25	42.67±0.14Aa	53.33±0.18Aa	43.33±0.06Aa	46.44±0.13a
45	30.67±0.15Ab	19.33±0.08Ab	14.00±0.32Ab	21.33±0.18b
Average	36.22±0.12A	37.11±0.12A	35.11±0.17A	

Remark: Numbers with different capital letters in the same row indicate significant differences in DHT treatment duration (p<0.05); Numbers with different lowercase letters in the same column indicate significant differences from the DHT temperature treatment (p<0.05).

Table 4. Effect of temperature and duration of Dry Heat Treatment on seed viability (%)

Dry Heat Treatment (°C)	Duration (hours)			Average
	6	12	18	
0	20.00±0.00Ba	8.00±2.00ABa	38.67±2.3Aa	22.22±1.44a
25	20.00±4.00Ba	36.00±1.73ABa	13.33±2.30Aa	23.11±2.68a
45	8.00±3.46Ba	12.00±3.46AB	6.67±0.58Ab	8.67±2.50b
Average	6.00±2.49B	18.67±2.40AB	19.33±1.73A	

Remark: Numbers with different capital letters in the same row indicate significant differences in DHT treatment duration (p<0.05); Numbers with different lowercase letters in the same column indicate significant differences from the DHT temperature treatment (p<0.05).

Table 5. Effect of temperature and duration of Dry Heat Treatment on vigor index (%)

Dry Heat Treatment (°C)	Duration (hours)			Average
	6	12	18	
0	50.67±1.03Aa	69.33±0.75Aa	57.33±1.10Aa	59.11±0.96a
25	65.33±0.13Aa	70.67±0.36Aa	73.33±0.59Aa	69.78±0.36a
45	53.33±0.41Ab	26.67±0.44Ab	21.33±0.22Ab	33.78±0.36b
Average	56.44±0.52A	55.56±0.52A	50.66±0.64A	

Remark: Numbers with different capital letters in the same row indicate significant differences in DHT treatment duration (p<0.05); Numbers with different lowercase letters in the same column indicate significant differences from the DHT temperature treatment (p<0.05).

Table 6. Effect of dry heat treatment interaction between temperature and duration on germination, seed viability, and vigor index

Dry Heat Treatment Interaction		Seed Germinatior (%)	Seed viability (%)	Vigor index (%)
Temperature (°C)	Duration(hours)			
0	**6**	35.33±0.08abc	20.00±0.00b	50.67±1.03abc
0	**12**	38.67±0.13abc	8.00±2.00c	69.33±0.75a
0	**18**	48.00±0.14a	38.67±2.30a	57.33±1.10a
25	**6**	42.67±0.14ab	20.00±4.00b	65.33±0.13a
25	**12**	53.33±0.18a	36.00±1.37a	70.67±0.36a
25	**18**	43.33±0.06ab	13.33±2.30bc	73.33±0.59a
45	**6**	30.67±0.15abc	8.00±3.46c	53.33±0.41ab
45	**12**	19.33±0.08bc	12.00±3.46c	26.67±0.44bc
45	**18**	14.00±0.32c	6.67±0.58c	21.33±0.22c

Remark: Numbers with different lowercase letters in the same column indicate significant differences from the combined temperature treatment and DHT duration (p<0.05).

Meanwhile, the interaction between a DHT temperature of 25 °C and a duration of 6, 12, and 18 hours was able to maintain germination, seed viability, and vigor index, as evidenced by the analysis of variance that showed no significant effect compared to the control (Table 6). This indicates that this temperature effectively maintains seed viability without causing significant damage and is in line with the statement of Ramdan et al. (2021) statement that physical treatment with dry heat does not affect soybean seed viability and does not interfere with plant physiological processes.

However, at 45 °C, germination was significantly decreased at 12 and 18 hours, indicating that this high temperature can damage seed viability if applied for too long and in line with research by Fauziyah et al. (2024) which states the results of their research that dry heat treatment at 45 °C for 12 and 24 hours does have the ability to suppress the population of *X. axonopodis* in soybean seeds. However, the temperature harms seed germination, so it has not had a natural effect on the vigor and viability of soybean seeds.

CONCLUSIONS AND SUGGESTIONS

This study found that the best dry heat treatment temperature to eliminate Xag in seeds was 25 °C and 45 °C. The longer the duration of DHT is given, the more the Xag population decreases. However, the higher the temperature, the more the germination rate, seed viability, and vigor index decreased. Therefore, the findings of this study recommend the best treatment to eliminate Xag and maintain soybean seed viability, namely the DHT technique with a temperature of 25 °C for 12 hours. Future research is needed by testing seeds using the growing-on test technique in soil media. This test is needed to determine the incidence of disease and the growth ability of soybeans in the nursery.

ACKNOWLEDGMENTS

Thanks to the Ministry of Education, Culture, Research, and Technology for funding the research in the Student Creativity Program in 2024. Thanks also to the University of Gunadarma for providing in-cash funding.

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