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Determination of mechanical damage on soybean seed by clorox soak test

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Abstract The Clorox soak test was assessed soybean seed damage, proving to be a comfortable and effective method for detection. Among different seed lots, the third lot exhibited the lowest percentage of damaged seeds. Notably, these seeds yielded seedlings with the highest root length, shoot length, and dry weight. Regarding damage levels, both undropped seeds and those dropped two times displayed the highest shoot length and seedling dry weight. Seed vigor assessed through accelerated aging that showed Lot 2 of soybean seeds had the highest vigor across all damage levels.

Keywords: *Glycine max* L., Damage seed, Physiological quality, Seed germination, Seed vigor

Introduction

Soybean (*Glycine max* L.) is globally recognized as an economically significant crop. It holds a prominent position as one of the largest sources of protein for animal feed and ranks as the world's second-largest supplier of vegetable oil. Furthermore, it plays a pivotal role as a primary raw material in numerous food processing industries (Huang, 2023). At present, an increasing number of farmers are shifting towards soybean cultivation. According to the World Agricultural Supply and Demand Estimates (WASDE) report, global soybean production for the 2022/2023 planting season is projected to reach 369.6 million metric tons (U.S. Department of Agriculture, 2023). Nonetheless, this production remains inadequate to satisfy the prevailing demand. In 2023, the global soybean processing industry is anticipated to expand from $78.78 billion to $84.91 billion, driven by the growing worldwide demand for soybean oil and processed foods (ReportLinker, 2023). Morever, soybean is not only directly consumed as food by humans and animals but also extensively used for the production of biodiesel. The versatility of soybeans across various industries highlighted their profound economic importance and nutritional value on a global scale.

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The environmental conditions, such as temperature, photoperiods, nutrient levels, rainfall, humidity, and soil moisture have significantly impacted on seed quality during the harvesting process. Seeds grown under different environmental conditions in the field may exhibit varying rates of deterioration. Moreover, vibrations occurring during harvesting and seed handling can also lead to seed damage, thereby affecting their quality. These effects may not immediately manifest but can result in latent effects, meaning that they do not immediately impact seed germination but cause seeds to deteriorate rapidly over time. Additionally, if seeds undergo mechanical stress during harvesting and transportation, such as breaking, cracking, or bruising, it can accelerate seed deterioration. Modern agricultural machinery has become integral in streamlining various processes involved in seed production, including harvesting, threshing, and seed conditioning. However, the mechanization of these processes can inadvertently cause damage to the seeds. Seed damage can occur due to factors such as impact, friction, compression, and other mechanical stresses, all of which can result in cracks, fractures, or wounds on the seed coat, particularly with leguminous plants. In many cases, these damages not only compromise the structure of the seeds but also lead to abnormal seedling germination and increased susceptibility to microbial attack (Shahbazi *et al*., 2011). Soybean seeds are sensitive to weather conditions and vulnerable to damage both during harvesting and in post-harvest processes, primarily due to their relatively thin seed coat. These damages have a detrimental effect on seed quality. Numerous researchers have substantiated that the majority of seed damage results from mechanical forces experienced during harvesting and transportation processes (Shahbazi *et al*., 2015). Recent studies have revealed that both harvesting and processing procedures frequently contribute to mechanical damage in seeds, presenting a significant challenge that leads to compromised seed quality (Gagare *et al*., 2014). This damage can have far-reaching consequences, affecting various critical aspects such as germination percentage, seedling establishment, and reproductive capacity. These factors collectively exert a profound influence on the overall potential crop yield. Consequently, the assessment of physiological seed quality emerges as a pivotal consideration in crop production. Employing healthy seeds tends to enhance yield productivity, whereas the use of seeds with damaged seed coats resulting from mechanized processes can lead to non-germination or the emergence of abnormal seedlings. Failure to assess seed damage before planting may necessitate replanting, thereby potentially increasing production costs. Thus, assessing seed damage before planting not only reduces risks but also enhances production efficiency. While visual inspection is one approach for seed damage assessment, it has limitations since it cannot detect internal damages. Another method, albeit one that takes longer to yield results, is the seed germination test. Additionally, various methods can be employed to check for damage (Jothityangkoon, 2002). The Clorox soak test stands out as a simple yet effective method. It requires only 10-15 minutes and can be conducted in both field and laboratory settings. This method is also applicable for detecting damaged seeds of other legumes, such as cowpeas or dicotyledon seeds similar in size to soybeans, that have incurred mechanical damage. Importantly, each lot of soybeans may exhibit varying degrees of damage.

The objective was to simulate mechanical damage on each lot of soybean seeds by introducing varying levels of damage. Subsequently, the extent of the damage was assessed using the Clorox soak test.

Materials and methods

The experiment was carried out from January to March 2023 at the Seed Technology Laboratory, located in the Chao Khun Thahan building within the Department of Plant Production Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang. In this study, we evaluated one cultivar of soybean (*Glycine max* L.), specifically 'Chiang Mai 60.' The experimental design employed was a 3 x 4 Factorial Experiment in Completely Randomized Design (CRD), with each treatment combination replicated four times. The two factors under investigation were as follows: Factor A: Three types of seed lots, denoted as lot 1 (harvested on January 10, 2023), lot 2 (harvested on February 10, 2023), and lot 3 (harvested on March 10, 2023). Factor B: Four levels of mechanical damage, encompassing non-dropped seeds, seeds dropped twice, seeds dropped four times, and seeds dropped eight times. The mechanical damage to soybean seeds was induced by dropping them from a height of 2 meters using a PVC pipe, with each level of damage corresponding to 0, 2, 4, or 8 drops (Taylor, 2013). The non-dropped seeds served as the control treatment. To account for potential seed loss during the dropping operation, a sample of 600 grams of soybean seeds was selected for each treatment. From each treatment, 200 seeds were randomly chosen. Following the seed-dropping procedure, the percentage of seed damage was determined through the Clorox soak test for each treatment. Additionally, the quality of the seeds and their vigor, including seedling growth, were assessed.

Seed coat damage by clorox soak method

A 5% Clorox solution (sodium hypochlorite) was prepared by mixing 5 mL of Clorox with 100 mL of distilled water. For each treatment, four replicates of 50 seeds were randomly counted. The seeds were soaked in the

Clorox solution for 10-15 minutes. After the specified time, the seeds were removed, and two categories were assessed: seeds with a wrinkled seed coat (indicating undamaged or slightly damaged seed coats) and seeds that had absorbed water, causing the seed coat to swell (indicating mechanically damaged seeds). The percentages of non-seed coat damaged and seed coat damaged seeds were calculated using the following formula:

$$\text{Non-seed coat damaged (\%)} = \left[\frac{\text{number of unseed coat damaged}}{\text{total seeds}}\right] \times 100$$

$$\text{Seed coat damaged (\%)} = \left[\frac{\text{number of seed coat damaged}}{\text{total seeds}}\right] \times 100$$

Seed germination percentage

Each treatment underwent germination using the between-paper method, with four replications for each treatment, each consisting of 50 seeds. Before testing, the seeds were disinfected with a 0.1% Clorox solution. Subsequently, the seeds were placed in a germination chamber at a constant temperature of 25°C. The number of germinated seeds was counted on the 5th day for the first count and on the 8th day for the final count, following the sample planting procedure as per ISTA guidelines (2019). Germination percentage was calculated using the following formula:

$$\text{Seed germination (\%)} = \left[\frac{\text{number of normal seedlings}}{\text{number of cultivated seeds}}\right] \times 100$$

Germination index

The germination index was determined using the between-paper method, following a procedure similar to the standard germination test. The number of seedlings that germinated as normal seedlings was counted on both the 5th and 8th days of seedlings, following the guidelines outlined in ISTA (2019). Subsequently, the obtained values were used to calculate the germination index using the following formula:

$$\text{Germination index} = \Sigma\left[\frac{\text{number of normal seedlings in each days}}{\text{number of days after cultivation}}\right]$$

Seedling root length

Ten seedlings from each replication of the germination test were randomly selected. The root length of each seedling was measured using a ruler, starting from the point where the root connects to the seedling's shoot and extending to the root tip. The average root length was then calculated using the following formula:

$$\text{Average of root length (cm)} = \left[\frac{\text{sum of seedlings root length}}{\text{number of seedlings}}\right]$$

Seedling shoot length

Ten seedlings from each replication of the germination test were randomly selected. The length of the root tip for each seedling was measured using a ruler, starting from the part adjacent to the seedling and extending to the apical part. The average shoot length was then calculated using the following formula:

$$\text{Average of root length (cm)} = \left[\frac{\text{sum of seedlings shoot length}}{\text{number of seedlings}}\right]$$

Seedling dry weight

The vigor of the seeds was assessed by using seedling dry weight as a criterion. It is a well-established principle that vigorous seeds tend to produce seedlings with higher dry weight. Seedlings from the root and shoot length test were subjected to drying in a hot air oven at 80°C for a duration of 24 hours. The average dry weight was then calculated using the following formula:

$$\text{Average of dry weight (mg/plants)} = \left[\frac{\text{sum of seedlings dry weight}}{\text{number of seedlings}}\right]$$

Seed vigor

The assessment of soybean seed vigor across different lots and damage levels was conducted using an accelerated aging method. For each treatment, 55 grams of soybean seeds were weighed and placed within a wire mesh, with a stand holding four meshes per seed lot. These were then positioned inside a plastic box containing water, with the water level approximately 2 centimeters below the sieve. The box was securely sealed with Scotch tape and subsequently placed in an accelerated aging chamber, set at a temperature of 41°C, for a period of 3 days. This procedure was in accordance with the protocol established by the Association of Official Seed Analysts (AOSA) in 1983. After the accelerated aging process, the soybean cultivars from each treatment were assessed for seed vigor expression by investigating both germination and the seed germination index.

Statistical analysis

To analyze the data, an Analysis of Variance (ANOVA) was performed, followed by a comparison of means using Duncan's New Multiple Range Test (DMRT) method. The analysis was conducted using the SAS (Statistical Analysis System) program.

Results

In the study of mechanical damage to soybean seeds using the Clorox soak test, we observed significant differences in soybean seed lots at a 99% confidence level. Among these lots, Lot 3 exhibited the highest percentage of non-seed coat damage (97.63%) and the lowest percentage of seed coat damage (2.38%). However, these differences were not statistically significant when compared to Lot 1. Conversely, Lot 2 had the lowest percentage of non-seed coat damage (93.75%) and the highest percentage of seed coat damage (6.25%). When considering the mechanical damage level alone, we found no statistical differences in the percentage of non-seed coat damage or seed coat damage. Exploring the interaction between soybean seed lot and mechanical damage level, we discovered no statistical differences in either non-seed coat damage or seed coat damage percentages. Seed germination was not significant differences in germination percentages in all soybean sed lots. Similarly, the interaction between soybean lot and mechanical damage level was also not statistically significant differences in seed germination percentages. Examining the germination index yielded similar outcomes, with no statistically significant differences observed among all soybean seed lots at different damage levels. Furthermore, the interaction between soybean seed lot and damage level did not produce any statistically significant differences in germination index (Table 1).

In this experimental study on soybean root lengths across different seed lots, significant variations were observed. Specifically, Lot 1 exhibited the shortest root length at 13.69 cm, while Lots 2 and 3 showed notably longer roots, measuring 15.22 cm and 16.48 cm, respectively. Interestingly, the root lengths did not significantly vary due to seed damage levels alone. Additionally, the interaction between seed lot and damage level did not significantly affect root length. In analyzing shoot lengths, we observed statistically significant variations among the seed lots (Factor A), across mechanical damage levels (Factor B), and in their interaction (A x B). Shoot length ranged from 10.32 cm in Lot 1 to 15.46 cm in Lot 3, demonstrating diverse responses to mechanical damage. Notably, non-dropped seedlings in Lot 3 had the highest shoot length, reaching 16.74 cm. Furthermore, a correlation was found between seedling dry weight and growth among the soybean seed lots. Lot 3 seedlings had the highest dry weight (54.06

mg/plant), followed by Lot 2 (49.38 mg/plant), and Lot 1 with the lowest (20.06 mg/plant). Mechanical damage levels had a significant impact on dry weight, especially at two drops. The interaction between seed lot and damage level also significantly influenced dry weight. For instance, twice-dropped seedlings in Lot 3 showed a dry weight comparable to undropped ones. In contrast, Lot 1 consistently had the lowest dry weight, regardless of damage level (Table 2).

Table 1. The percentage of non-seed coat damaged and seed coat damaged from the clorox soak test, percent germination and germination index after 0, 2, 4, and 8 drops of soybean seeds

Factors		Non- seed coat damaged [1/] (%)	Seed coat damaged [1/] (%)	Germination (%)	Germination index
Seed lot (A)					
Lot 1		96.63 a	3.38 b	97.63	9.76
Lot 2		93.75 b	6.25 a	99.13	9.91
Lot 3		97.63 a	2.38 b	98.63	9.86
Mechanical damage level (B)					
non-dropped		96.33	3.67	99.17	9.91
2 times dropped		95.83	4.17	97.50	9.75
4 times dropped		96.33	3.67	98.67	9.87
8 times dropped		95.50	4.50	98.50	9.85
A x B					
Lot 1	non-dropped	96.50	3.50	99.00	9.90
	2 times dropped	95.50	4.50	97.00	9.80
	4 times dropped	97.50	2.50	96.50	9.65
	8 times dropped	97.00	3.00	98.00	9.80
Lot 2	non-dropped	95.50	4.50	99.00	9.90
	2 times dropped	93.50	6.50	98.00	9.80
	4 times dropped	94.50	5.50	100.00	10.00
	8 times dropped	91.50	8.50	99.50	9.95
Lot 3	non-dropped	97.00	3.00	99.50	9.95
	2 times dropped	98.50	1.50	97.50	9.75
	4 times dropped	97.00	3.00	99.50	9.95
	8 times dropped	98.00	2.00	98.00	9.80
F-test					
A		**	**	ns	ns
B		ns	ns	ns	ns
A x B		ns	ns	ns	ns
C.V. (%)		2.19	54.65	2.11	2.11

ns and **: not significantly different, significantly different at 95%, and 99% level, respective.
[1/]Different uppercase letters in same vertical are significantly different by method of Duncan's New Multiple Range Test (DMRT).

Table 2. The root length, shoot length and dry weight affected 0, 2, 4 and 8 drops on soybean seedlings

Factors		Root length[1/] (cm)	Shoot lenght[1/] (cm)	Dry weight[1/] (mg/plants)
Seed lot (A)				
Lot 1		13.69 b	10.32 c	20.06 c
Lot 2		15.22 a	12.85 b	49.38 b
Lot 3		16.48 a	15.46 a	54.06 a
Mechanical damage levels (B)				
non-dropped		14.98	14.43 a	42.00 a
2 times dropped		15.87	13.29 b	43.50 a
4 times dropped		14.41	11.64 c	39.58 b
8 times dropped		15.26	12.15 c	39.58 b
A x B				
Lot 1	non-dropped	14.86	12.09 c	19.50 e
	2 times dropped	13.41	8.77 e	19.50 e
	4 times dropped	12.24	9.65 de	20.75 e
	8 times dropped	14.26	10.76 cd	20.50 e
Lot 2	non-dropped	13.51	14.46 b	51.00 c
	2 times dropped	16.79	15.61 ab	55.25 ab
	4 times dropped	15.40	10.78 cd	46.25 d
	8 times dropped	15.20	10.56 cd	45.00 d
Lot 3	non-dropped	16.57	16.74 a	55.50 ab
	2 times dropped	17.41	15.50 ab	55.75 a
	4 times dropped	15.61	14.50 b	51.75 bc
	8 times dropped	16.33	15.13 ab	53.25 abc
F-test				
A		**	**	**
B		ns	**	*
A x B		ns	**	*
C.V. (%)		12.14	8.80	6.09

ns, * and **: not significantly different, significantly different at 95%, and 99% level, respective.
[1/]Different uppercase letters in same vertical are significantly different by method of Duncan's New Multiple Range Test (DMRT).

Upon examining seed vigor using the accelerated aging method, we found that soybean seeds from Lot 2 exhibited the highest percentage of germination (98.13%) and germination index (9.81). These values did not significantly differ from the germination percentage and germination index observed in seeds from Lot 3. In contrast, seeds from Lot 1 displayed the lowest germination percentage at 90.88% and the lowest germination index value of 9.09. Regarding seed damage levels, no statistically significant differences were observed in seed vigor in terms of germination percentage and germination index. However, when investigating the interaction between soybean seed lot and the level of damage caused by machinery, we observed statistically significant differences in the germination percentage and germination index of soybean seeds at a 95% confidence level. Specifically, seeds from Lot 2, subjected to two drops, and seeds from Lot 3, subjected to four drops, exhibited the highest germination percentages at 100% and the highest germination index at 10. These values did not significantly differ between the two damage levels. Conversely, seeds from Lot 1, subjected to eight drops, displayed the lowest germination percentage at 88.50% and the lowest germination index at 8.85, which was not statistically different from seeds in Lot 1 that were undropped or dropped four times (Table 3).

Table 3. The percentage of germination and germination index affected 0, 2, 4 and 8 drops on soybean seeds after accelerated aging

Factors		Germination[1/] (%)	Germination index[1/]
Seed lot (A)			
Lot 1		90.88 b	9.09 b
Lot 2		98.13 a	9.81 a
Lot 3		96.50 a	9.65 a
Mechanical damage levels (B)			
non-dropped		94.83	9.48
2 times dropped		95.50	9.55
4 times dropped		95.68	9.57
8 times dropped		95.97	9.47
A x B			
Lot 1	non-dropped	90.00 de	9.00 de
	2 times dropped	94.00 bcd	9.40 bcd
	4 times dropped	91.00 de	9.10 de
	8 times dropped	88.50 e	8.85 e
Lot 2	non-dropped	98.50 ab	9.85 ab
	2 times dropped	100.00 a	10.00 a
	4 times dropped	96.00 abc	9.6 abc
	8 times dropped	98.00 ab	9.80 ab
Lot 3	non-dropped	96.00 abc	9.60 abc
	2 times dropped	92.50 cde	9. 25 cde
	4 times dropped	100.00 a	10.00 a
	8 times dropped	97.50 ab	9.75 ab
F-test			
A		**	**
B		ns	ns
A x B		*	*
C.V. (%)		3.05	3.05

ns, * and **: not significantly different, significantly different at 95%, and 99% level, respective.
[1/]Different uppercase letters in same vertical are significantly different by method of Duncan's New Multiple Range Test (DMRT).

Discussion

The Clorox soak test, used to assess seed coat damage in soybean seeds, is a quick and effective method. It offers convenience and comfort as it only requires 10-15 minutes for examination, making it a suitable choice for inspections in both field and laboratory settings (Duangpatra, 1986). Sodium hypochlorite, commonly used to assess damage by causing cracked hulls in soybeans, has been recognized as a suitable test for quantifying mechanical damage in soybean seeds (VanUtrecht *et al*. 2000). Several factors, including water quantity, genetic variability, and harvesting time, significantly influence the susceptibility of seeds to physical damage (Silva *et al*., 2011). Physical damage to seeds can occur at various stages, beginning with planting, maturation, harvesting, conditioning, and storage (McDonald and Jr, 1985). Pre-harvest environmental conditions also play a pivotal role in determining the initial quality of seeds. When seeds suffer damage during quality improvement processes, handling, and transportation, their quality deteriorates more rapidly. Each seed lot may exhibit different initial quality levels and susceptibility to damage. In one of our observations, Lot 3 of soybean seed cultivar Chiangmai 60 displayed the highest percentage of good seeds and the lowest percentage of seed coat damage. Furthermore, these seeds produced seedlings with the longest root length, shoot length, dry weight, and seed vigor. Concerning damage levels, non-dropped and two-times dropped soybean seeds displayed the highest shoot length and seedling dry weight. However, Lot 2 of soybean seeds for all damage levels exhibited the highest seed vigor. Notably, there was no statistical difference between Lot 2 and Lot 3 of soybean seeds. These results suggest a clear correlation between seed strength and seedling growth. Physical damage directly impacts seed strength, subsequently influencing the potential for seedling growth. The damage primarily affects the seed coat, which serves to protect the embryo inside the seed from unfavorable environmental conditions. It also prevents the absorption of water and air and guards against infestations by pathogens and insects (Chulaka, 1995; Aekaraj, 2003). Proper soybean seed storage is essential, with a moisture content of approximately 15% being optimal. Seeds should not drop below 11% moisture content, as excessively dry seeds can become brittle and prone to cracking during harvesting. Mechanical harvesters have been linked to increased seed damage and breakage, worsening seed viability (Albaneze *et al*., 2018). Harvesting seeds with lower moisture content can further raise the percentage of broken and mechanically damaged seeds. Earlier studies, such as Neves *et al*. (2016) noted that the percentage of mechanical soybean seed damage is higher in the initial processing stage (hopper) but significantly decreases during pre-cleaning. The pre-cleaning process involves the removal of broken seeds, as they tend to separate. Mechanical seed damage percentages rise again after passing through dryers but decline after passing through sieves. Gagare *et al.* (2014) have demonstrated that seed management during harvesting and processing often leads to increased mechanical seed injury, a significant factor in low seed quality. Soybean seeds are particularly sensitive to mechanical damage due to their thin seed coat, a finding consistent with Goli *et al*. (2016). Their study found that the soybean cultivar L-17 is susceptible to deterioration after processing, and seed damage increases with the impact speed velocity. Soybean seeds are prone to mechanical damage during harvesting, threshing, and storage. Seed coats that have suffered mechanical damage lose their germination capacity more rapidly than undamaged seeds, leading to decreased seedling percentages. When examining seed germination and germination index, our study found no statistical difference among all lots of soybean seed cultivar Chiangmai 60 at various damage levels. This is in line with Chulaka (1999), who observed that although seeds damaged by mechanical means may display visible cracks, their germination in immediate tests may not differ from that of normal seeds. However, the germination of these damaged seeds tends to decrease rapidly during storage. Mechanical damage can have both immediate and latent effects, resulting in reduced seed strength over time (Souza *et al*., 2009).

In summary, the Clorox soak test is a reliable method for detecting damage in soybean seeds, offering both convenience and effectiveness. This method provides a practical and effective approach for farmers to evaluate seed quality prior to planting, thereby reducing the risk of abnormal seedling growth in the field. Among the seed lots, Lot 3 of soybean seeds exhibited the lowest percentage of damaged seed coats, resulting in seedlings with the highest root length, shoot length, dry weight, and seed vigor. Regarding the level of damage, undropped and two-times dropped soybean seeds consistently displayed the highest shoot length and seedling dry weight. In terms of seed vigor assessed through accelerated aging, Lot 2 of soybean seeds consistently demonstrated the highest seed vigor across all damage levels. If seed damage exceeds 10%, it is recommended to adjust agricultural machinery, including harvesters and seed conditioners, to minimize further damage.

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