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Ultrafine Bubbles Water priming to improve viability and vigor of bean (*Phaseolus vulgaris*) seeds

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Abstract

Priming is a physiological technique of hydration of seed to improve metabolic processes before germination to accelerate germination and seedling growth under normal and stressful conditions. This research is aimed to study the pretreatment using ultra-fine bubble (UFB) water to increase the viability and vigor of seeds before planting (pre-planting) and during storage (pre-storage). This research was conducted at the Laboratory of Seed Quality Testing, IPB University, Indonesia. The research consisted of two experiments, i.e., UFB water priming to increase the viability and vigor of bean seeds and their storability after priming. The first experiment was arranged using a completely randomized design with a combination of seed lot treatments (L1: initial germination percentage (GP) about 80%, L2: initial GP around 70%, and L3: initial GP around 60%) with priming (P0: no priming/control, P1: soaked in distilled water for 60 minutes, P2: distilled water for 120 minutes, P3: 8 ppm UFB water for 60 minutes, P4: 8 ppm UFB water for 120 minutes, P5: 20 ppm UFB water for 60 minutes, P6: UFB water 20 ppm for 120 minutes). The second experiment was arranged using a nested design with the main factor being the storage room condition and priming as the second factor nested in the main factor. The storage room condition factors consisted of KM: room conditions (temperature 28±5^{0}C, RH 73±7%) and AC: air-conditioned room (18±2^{0}C, RH 61±7%). The priming factor consisted of two levels, namely P0: without priming and P1: priming with 20 ppm UFB water soaked for 120 minutes. The results showed that priming treatment with UFB water 20 ppm for 120 minutes as a pre-planting treatment could increase the viability of bean seeds, particularly for seeds with low initial viability, and primed bean seeds with an initial viability of approximately 80% were able to retain their viability for 16 weeks of storage in an air-conditioned room.

Keywords: germination, pre-planting, pre-storage, seed storability, seed quality

Introduction

Quality seed is one of the keys to the success of agricultural production. Bean seed (*Phaseolus vulgaris* L.) is one of the essential commercial horticultural crops with high market demand, so good quality seed for planting materials is required. Bean seeds undergo a period of storage before reaching the farmers for planting. Good handling during storage will maintain the storability of the seeds, but the longer the seeds are stored, the seeds will deteriorate or decline so that their viability of the seeds will decrease. The process of seed deterioration can be classified into chronological decline caused by time factors and physiological decline caused by storage environmental factors.

Invigoration is one way to increase seed viability and vigor. Ilyas (2012) stated that invigoration could be in the form of osmoconditioning priming and matriconditioning. Osmoconditioning priming is a pre-planting treatment developed to increase germination. Mohajeri et al. (2016) showed that the invigoration of bean seeds is carried out by immersing the seeds in osmotic solutions such as $CaCl_2$, KCl, NaCl, and PEG. The results have shown that beans seeds soaked for 6 hours in $CaCl_2$ had the highest percentage of germination at 95% while the control was 84%. The research of Yuanasari et al. (2015) exhibited that black soybean seeds that were stored for 22 months in a storage room at a temperature of 12±2ºC with a humidity of ±60% had a germination rate of 76.67%

after osmoconditioning treatment using PEG-6000 for 12 hours germination rate increased rate to 86.00%.

Invigoration is used not only as a pre-planting treatment but also as a pre-storage treatment to maintain seed vigor during storage. Invigoration is used for marketable seeds to increase germination uniformity in the field. Waqas et al. (2019) stated that priming is a physiological technique of hydration and seed drying to improve metabolic processes before germination to accelerate germination, seedling growth, and plant yields under normal and stressful conditions. Nawaz et al. (2013) explained that the increase in seed germination after priming treatment was caused by the cell cycle activation process and reduced the endosperm covering so that the seeds remained in the second phase of imbibition and were ready to germinate.

Research by Utami et al. (2013) on long beans seeds invigorated by immersion for 2 hours in water (hydropriming), $CaCl_2$, KNO_3, and ascorbic acid can increase seed vigor index and can be maintained until the end of short-term storage up to 15 weeks both in air conditioning room and room temperature. Likewise, the results of Yan (2017) on Chinese cabbage seeds before priming had a germination rate of 69.4±2.4%, after hydropriming and stored for six months at 4^0C germination rate increased to 78.2±2.5, while after storage at 20^0C the germination rate was still 76.2±3.4% and at a storage temperature of 30℃ germination rate was 74.8±2.2%.

Hydropriming by giving constant water bubbles can also increase the viability and vigor of rice seeds. High viability after hydropriming can be maintained for up to 60 days of storage in aluminum foil packaging at a low temperature of -4 ℃ (Wang et al., 2018). New technology with great potential for increasing seed viability and vigor is ultra-fine bubbles (UFB) water in the form of micro and nano-sized fine bubbles water (Liu et al., 2016). UFB water has been widely studied for improving seed viability and vigor in several commodities, including soybean (Purwanto et al., 2019), *Gmelina arborea* (Siregar et al., 2020), and white jabon (Fata et al., 2020) seeds.

Numerous researches on priming was conducted by using UFB water to treat pre-planting seeds instead of pre-stored. UFB water increased seed viability and vigor, including invigoration with the hydropriming technique. This study aims to use UFB water to improve seed quality (viability and vigor) and evaluate its effect after the beans are stored.

Material and Methods

Location

The research was carried out from April 2021 to June 2022 at the Seed Quality Testing Laboratory, Leuwikopo Experimental Field, Department of Agronomy and Horticulture, Faculty of Agriculture, and Biosystem Engineering Laboratory, Department of Mechanical and Biosystem Engineering, Faculty of Agricultural Engineering and Technology, IPB University, Bogor, Indonesia.

Materials and Methods

The material used was “Rofi” variety beans with three lots of different quality seeds produced by PT East West Seed Indonesia, which consisted of lots with germination capacity of about 80% (L1), 70% (L2), and 60% (L3). Aluminum foil packaging is used to store seeds after priming. The equipment required is seed germination equipment (eco germinator type IPB 72-l, plastic, and stencil paper as germination media), equipment for determining seed moisture content (oven 130^0C, analytical balance, porcelain cup), and equipment for making UFB water (UFB water generator 9FZIN-10, IDES and pure oxygen with concentrations of 8 ppm and 20 ppm).

Experiment 1. Priming to Increase Viability and Vigor of Bean Seeds

The experiment was arranged in a completely randomized design with one factor with a combination of seed lot treatments (L1: initial GP about 80%, L2: initial GP around 70%, and L3: initial GP around 60%) and priming type (P0: no priming/control, P1: soaked in distilled water for 60 minutes, P2: distilled water for 120 minutes, P3: UFB water 8 ppm for 60 minutes, P4: UFB water 8 ppm for 120 minutes, P5: UFB water 20 ppm for 60 minutes, P6: UFB water 20 ppm for 120 minutes). The combinations obtained were 21 and repeated four times so that there were 84 experimental units. Two hundred bean seeds from each seed lot were soaked in 250 ml of distilled water, UFB water 8 ppm, and 20 ppm according to the priming treatment that had determined for 60 minutes and 120 minutes. UFB water 8 ppm used oxygen from the room room, while UFB water 20 ppm used pure oxygen injection. Seeds that have been treated are dried to reach the initial moisture content for 7×24 hours at a temperature of 18±2˚C and humidity of 61±7%. Seeds were germinated using the between paper method at seed germinator type IPB72-l at 25±5˚C and RH 70±7% in the laboratory. The growth and time of emergence of seeds from treated seeds were also tested in the field by planting one seed in

each planting hole.

Experiment 2. The Storability of Bean Seeds After Priming

The experiment was arranged using a nested design, with the main factor being the storage room condition and priming as the second factor nested in the main factor. The storage room condition factors consist of KM: room temperature (temperature 28±5^{0}C, RH 73±7%) and AC: air-conditioned room condition (18± 2^{0}C, RH 61±7%). The priming factor consisted of two levels, namely P0: without priming and P1: priming with UFB water 20 ppm soaked for 120 minutes. The treatment was repeated four times so that there were 16 experimental units for each observation. The treatment was applied to seed lots with an initial GP of around 80%. The storage period was for 16 weeks, and the seeds' viability and vigor were tested every two weeks.

Statistical Analysis

Variance analysis was carried out using Statistical Analysis System (SAS) Enterprise Guide 7.1 at α=0.05; the Duncan test was used for further analysis.

Result and Discussion

Priming to Increase Viability and Vigor of Bean Seeds

The results of the analysis of variance showed that the combination of treatments between seed lots and priming had a very significant effect on moisture content (MC), germination percentage (GP), vigor index (VI), maximum growth potential (MGP), Seedling growth rate (SGR), growth speed (GS), T_{50}, normal seedling dry weight (NSDW), electrical conductivity (EC), mean emergence time (MET) and field emergence (FE) (Table 1).

After priming, the seeds were dried to minimize seed moisture content. The drying process lasted 24 hours and seven times 24 hours. Drying for 24 hours after priming for 60 minutes resulted in a seed moisture content of approximately 22-29%, whereas priming for 120 minutes resulted in a moisture content of approximately 30-35%. The still-high moisture content needs drying to attain the initial moisture content of seeds before priming, which is 11-12%. At all initial viability levels, 7 x 24 hours of drying reduced seed moisture content to 10-12%, comparable to seed moisture content without priming.

According to the germination percentage parameter, seeds with high initial viability or lot 1 (initial germination percentage 82.5%) were insensitive to priming treatment; even priming with distilled water and UFB water 20 ppm for 60 minutes lowered germination percentage (69.50% and 71.00%, respectively). Priming seeds with medium initial viability or lot 2 (initial germination percentage 77.5%) did not significantly affect seed viability, and lowered germination percentage after priming with distilled water for 120 minutes (64.5%). Priming with UFB water 20 ppm for 120 minutes significantly improved the germination percentage of seed lots with low initial viability or lot 3 (from 69.5% to 90.50%). (Table 2). This outcome is consistent with Siregar's (2020) research findings. Invigoration or priming of *Albizia chinensis* with high initial viability did not improve

Table 1. Recapitulation of variance of priming effect on the quality of three bean seed lots that have different initial viability

No.	Variable	Lot×Priming	CV
1	Moisture content (MC)	**	0.97
2	Germination percentage (GP)	**	8.80
3	Maximum growth potential(MGP)	**	4.83
4	Seedling growth rate (SGR)	ns	14.77
5	T_{50}	**	3.62
6	Vigor index (VI)	**	14.98
7	Germination speed (GS)	**	14.69
8	Normal seedling dry weight (NSDW)	**	11.75
9	Electrical conductivity (EC)	**	10.42
10	Mean emergence time (MET)	ns	6.29
11	Field emergence (FE)	**	10.97

Note: * = significant at P < 0.05. ** = significant at P < 0.01; ns = not significantly different according to the F test with 95% confidence level.

Table 2. Germination percentage, maximum growth potential, normal seedling dry weigh, and seedling growth rate in response to various priming treatments.

Seeds lot	Priming	GP	MGP	NSDW	SGR
		(%)	(%)	(g)	(g/KN)
L1	P0	82.50 abcd	93.00 abc	2.63 bcd	0.064
	P1	69.50 efgh	90.00 abcd	2.12 fg	0.061
	P2	82.50 abcd	96.00 a	2.82 ab	0.069
	P3	80.00 bcde	94.00 abc	2.59 bcde	0.065
	P4	80.00 bcde	95.00 ab	2.70 bc	0.068
	P5	71.00 efgh	88.00 bcd	1.97 fg	0.056
	P6	92.00 a	95.50 ab	3.22 a	0.069
L2	P0	77.50 cdef	91.00 abcd	2.49 bcdef	0.064
	P1	69.50 efgh	84.50 de	2.17 efg	0.062
	P2	64.50 gh	79.50 e	2.12 fg	0.066
	P3	72.00 defgh	87.00 cd	2.41 bcdefg	0.068
	P4	76.00 def	88.00 bcd	2.48 bcdef	0.066
	P5	70.50 efgh	91.00 abcd	2.08 fg	0.059
	P6	87.00 abc	94.50 abc	2.82 ab	0.065
L3	P0	69.50 efgh	96.00 a	2.05 fg	0.059
	P1	77.50 cdef	92.50 abc	2.33 cdefg	0.060
	P2	61.00 h	89.00 abcd	1.48 h	0.056
	P3	70.50 efgh	89.50 abcd	2.19 defg	0.063
	P4	67.00 fgh	90.00 abcd	2.02 fg	0.061
	P5	73.00 defg	93.50 abc	2.12 fg	0.058
	P6	90.50 ab	93.50 abc	2.66 bc	0.059

Note: Values within the same column followed by the same letter are not significantly different based on the DMRT. GP = germination percentage. MGP = maximum growth potential. NSDW = Normal seedling dry weight. SGR = Seedling growth rate

germination percentage, whereas priming with UFB water increased germination percentage in seeds with poor initial viability.

All priming treatments failed to raise the maximum growth potential in all lots of bean seeds, and in the case of lot 2, priming with distilled water for 120 minutes decreased maximum growth potential values (Table 2). The maximum growth potential number was derived from normal and abnormal germination computation. Therefore, even though it was high it did not influence the germination percentage or seed vigor. In contrast, priming with UFB water 20 ppm for 120 minutes raised normal seedling dry weight in lots 1 and 3, but had no significant effect on lot 2.

Priming can boost seed vigor. The improvement in seed vigor was demonstrated by the vigor index, T50, growth speed, and electrical conductivity (Table 3). Priming with UFB water 8 ppm and UFB water 20 ppm for 120 minutes raised the vigor index in all seed lots examined, however priming with UFB water 20 ppm for 60 minutes had no effect on seed lots with high initial viability. All seed lots exhibited an increase in T50 following priming with UFB water 8 ppm for 120 minutes, UFB water 20 ppm for 60 minutes, and 120 minutes. Priming with distilled water for 120 minutes, UFB water 8 ppm for 60 minutes, and aqua dest for 60 minutes enhanced T50 in lots 2 and 3, whereas priming with aquadest for 60 minutes increased T50 in the seeds with the lowest quality. After priming with UFB water 20 ppm for 120 minutes, growth speed was increased in lots 1 and 2. White jabon seeds were able to improve growth speed, consistent with the findings of Fata's research (2020) on UFB.

Significantly different from the control, seed priming with UFB water 20 ppm reduced the electrical conductivity value in lot 1. The lower the electrical conductivity score implies that the viability and vigor of the seeds are still high. The priming treatment in lots 2 and 3 had a favorable effect on the electrical

conductivity parameter. Except for priming with UFB water 8 ppm for 60 minutes, all priming procedures in lot 2 and lot 3 reduced the electrical conductivity value compared to the control. The field emergence of seeds revealed that none of the priming treatments increased field emergence in any of the seed lots that were examined.

Priming with UFB water 20 ppm for 120 minutes was the treatment that improved seed quality the most at the three initial viability levels examined (vigor index, T50, and electrical conductivity; see Table 3). High seed vigor refers to the capacity of seeds to survive in suboptimal environments. Very fine UFB water can reach the seeds through the seed coat, enhancing seed respiration. According to Liu et al. (2016), as more oxygen is transported into the seed, the seed's respiration will increase, allowing it to spend more energy for germination. In addition, Gomes and Garcia (2013) demonstrated that UFB water generates hydroxyl radicals that can induce the development of reactive oxygen species (ROS) in seeds, which act as physiological regulators for germination signaling and can enhance gibberellin levels in seeds.

Shelf Life of Bean Seeds After Priming

After priming and prior to storage, the initial moisture content of seeds is approximately 13% (Figure 1). According to government regulations, this moisture content are not safe for storing bean seeds which is 11% (Ministry of Agriculture, 2019). This increased moisture content led to decreased seed viability and vigor during storage of 16 WAS (weeks after storage) relative to the control (Figures 2, 3, and 4). The high initial moisture content enables the seeds to have a high respiration process.

The germination percentage of seeds without priming could be maintained up to 16 WAS both in the room and in the air-conditioned room (>80%), however, seeds with UFB water 20 ppm for 120 minutes decreased the germination percentage. However, the germination percentage treated with priming and stored in a room with air conditioning increased over time. During the storage of 16 WAS, the germination percentage of seeds treated with priming and stored at room temperature continued to decline (Figure 2). This may arise because the seed lot utilized

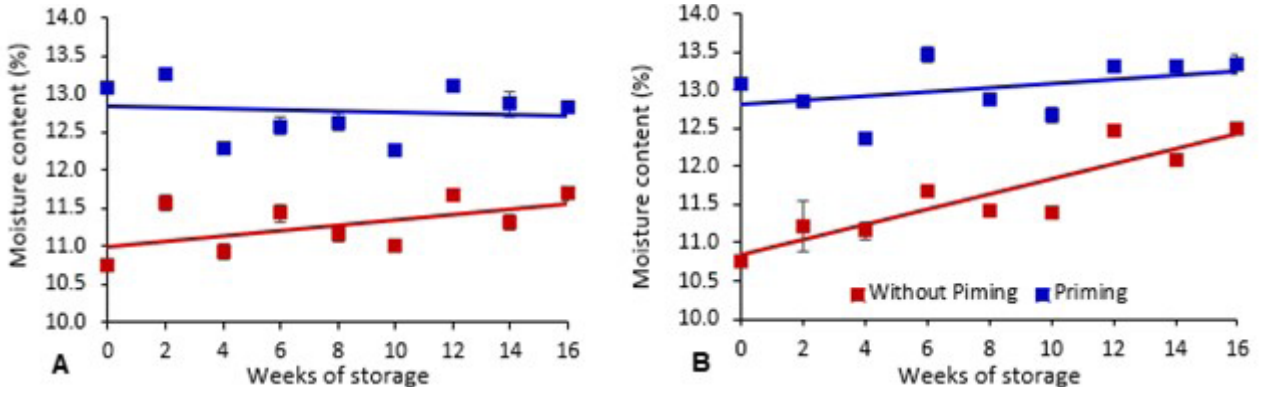

Figure 1. Moisture content of bean seeds without priming and priming as a response to the conditions of the seed storage space during 16 weeks of storage: (A) air-conditioned room, (B) room temperature

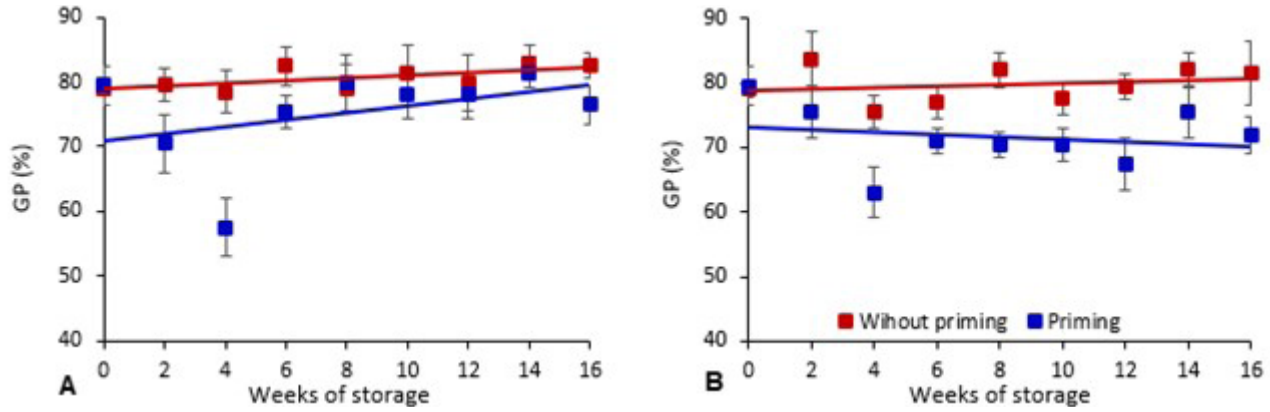

Figure 2. Germination of bean seeds without priming and priming in response to the condition of the seed storage space during 16 weeks of storage: (A) air-conditioned room, (B) room temperature

Table 3. Vigor index, T_{50}, growth speed, electrical conductivity, mean emergence time, and field emergence in response to various priming treatments

Lot Benih	Priming	VI	T_{50}	GS	EC	MET	FE
		(%)	(day)	(%KN/etmal)	(μS cm^{-1} g^{-1})	(day)	(%)
L1	P0	46.95 cde	4.08 b	19.80 bcde	24.01 bc	5.38 a	59.00 abcde
	P1	44.90 cdef	4.09 b	19.70 bcde	22.31 bcde	5.05 a	63.00 abcd
	P2	67.33 a	3.99 bc	19.00 bcde	20.66 cdef	4.95 a	51.50 efg
	P3	53.50 bc	3.91 bcd	21.28 abcd	21.02 cdef	5.18 a	64.00 abc
	P4	68.00 a	3.67 efgh	21.93 abc	21.55 cde	5.05 a	63.00 abcd
	P5	54.78 bc	3.48 hi	22.15 abc	21.81 cde	5.25 a	67.50 a
	P6	71.03 a	3.01 l	25.48 a	17.52 f	5.00 a	65.00 ab
L2	P0	38.50 def	4.10 b	17.48 cdef	28.70 a	5.30 a	53.00 defg
	P1	50.23 bcd	4.08 b	13.63 f	22.48 bcde	5.18 a	53.00 defg
	P2	42.38 cdef	3.83 cdef	16.48 def	20.89 cdef	5.00 a	37.00 h
	P3	46.75 cde	3.87 cde	17.55 cdef	20.89 cdef	5.08 a	55.50 bcdefg
	P4	67.56 a	3.59 gh	18.80 bcde	21.97 bcde	4.98 a	54.50 cdefg
	P5	52.30 bc	3.22 jk	19.70 bcde	20.63 cdef	4.83 a	58.50 abcdef
	P6	62.43 ab	3.10 kl	22.53 ab	19.94 def	5.05 a	56.50 bcdefg
L3	P0	32.82 f	4.33 a	17.48 cdef	28.77 a	5.28 a	51.50 efg
	P1	50.23 bcd	3.77 defg	16.28 ef	23.69 bcd	5.03 a	48.00 g
	P2	35.92 ef	3.58 gh	17.33 cdef	23.96 bc	5.18 a	48.50 fg4
	P3	46.50 cde	3.63 fgh	19.35 bcde	25.78 ab	5.00 a	53.00 defg
	P4	45.45 cde	3.68 efgh	17.95 bcdef	23.21 bcd	4.90 a	49.00 efg
	P5	60.83 ab	3.34 ij	19.63 bcde	22.11 bcde	5.25 a	55.00 bcdefg
	P6	61.55 ab	3.04 kl	21.43 abc	18.72 ef	4.93 a	54.00 cdefg

Note: Values within the same column followed by the same letter are not significantly different based on the DMRT. VI = vigor index, T_{50} = time required to 50% germination, GS = growth speed, EC = electrical conductivity, MET = Mean emergence time, FE = Field emergence.

possesses high initial viability and vigor (around 80% initial germination percentage). According to research by Powell et al. (2000), priming with water immersion for 28 hours on cauliflower seeds with high initial vigor has a negative effect on seed shelf life, however, priming on seeds with low vigor can extend seed shelf life.

Based on T_{50} (50% normal germination time), the vigor of priming and control seeds stored in both the air-conditioned and room temperature did not differ substantially (Figure 3). Intriguingl the vigor index of priming seeds increased throughout storage in both storage conditions (Figure 4), whereas the vigor index of non-primed seeds decreased during storage in room temperature circumstances. After being stored in an air-conditioned room, priming and non-priming seeds had the same vigor index.

Seed quality can be maintained during storage if the seeds are stored under optimal conditions. The storage of priming seeds at high temperatures and RH accelerates seed respiration, utilizing food reserves. The seed quality of beans stored in the room temperature was lower than beans that are not primed. The decline in viability and vigor the room temperature-stored bean stored bean seeds could be induced by high temperature and relative humidity (28±2ºC, 73±7%). According to Wang et al. (2018), situations of high RH can result in seed degeneration due to an increased lipid peroxidation. After priming, seed storage in an air-conditioned room is preferable to storage at room temperature. According to Adhinugraha et al. (2022), the true seed of shallot (*Allium ascalonicum* L.) stored in an air-conditioned environment following revitalization can be maintained for 14 weeks. This study revealed that priming and storing bean seeds in an air-conditioned room boosted their quality despite their high moisture content.

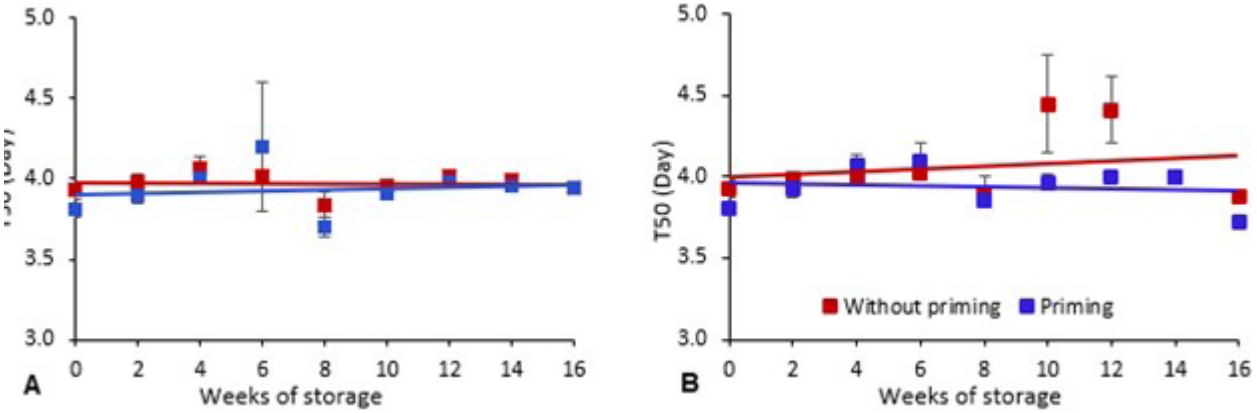

Figure 3. T50 of bean seeds without priming and priming as a response to the condition of the seed storage space during 16 weeks of storage: (A) air-conditioned room, (B) room temperature

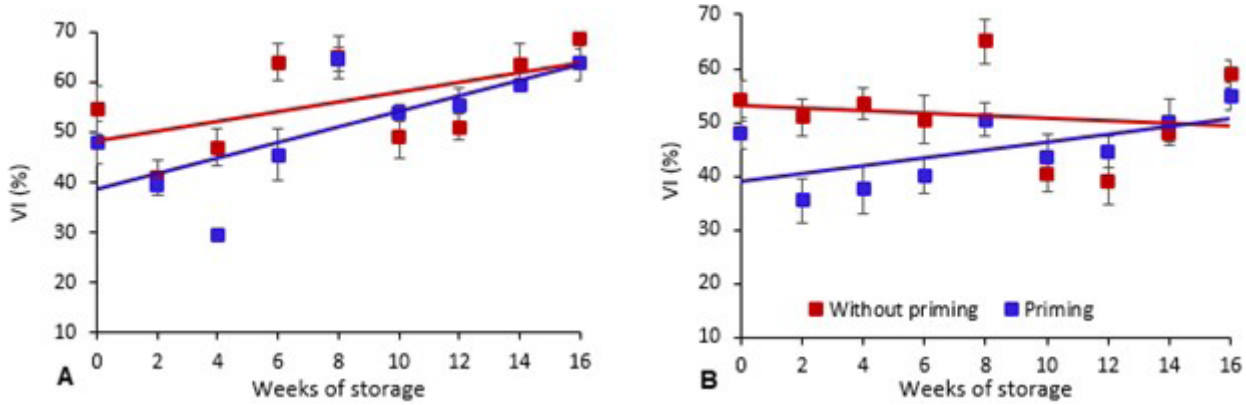

Figure 4. Vigor index of bean seeds without priming and priming as a response to the condition of the seed storage space during 16 weeks of storage: (A) air-conditioned room, (B) room temperature

Ultra-fine bubble water provides oxygen to the seeds, hence boosting seed respiration. When seeds are stored, respiration will continue, resultdeclining seed viability. The reduced viability and vigor of seeds in priming treated with UFB water 20 ppm for 120 minutes may also be owing to the influence of extremely high ROS, which destroys the seeds' macromolecules. ROS can induce oxidative damage to macromolecules, DNA damage, and DNA splitting/ shortening, according to Tomizawa et al. (2005). Ishibashi et al. (2009) discovered that excessive concentrations of reactive oxygen species (ROS) might act as damaging substances; nevertheless, under optimal conditions, ROS will play an essential role in relaxing cell walls and signaling crucial plant growth activities.

A study in rice, lettuce and radish reported that priming shortened the seed shelf life, (Hill et al. 2008, Hussein et al. 2015, Malek et al. 2019). While other researchers found that priming boosted and prolonged the shelf life of seeds of *Digitalis purpurea*, *Rhododendron griersonianum*, long beans, chicory, rice, and shallots (Butler et al. 2009; Wood and Hay 2010; Utami 2013; Yan 2017; Wang et al. 2018; Adhinugraha et al. 2022). This results demonstrate that priming with UFB water 20 ppm for 120 minutes enhanced the viability of bean seeds stored in an air-conditioned environment until week 16. Meanwhile, priming bean seeds stored at room temperature was insufficient to preserve the seeds' shelf life until 16 WAS. The failure to lower the moisture content until they are safe for storage is believed to be the leading cause of the deterioration in seed quality following priming. If seeds treated with priming are not followed by a decrease in safe storage moisture content, seed deterioration will be accelerated. In order to preserve the viability and vigor of the primed seeds during storage, the seeds should preferably be dried and stored at low humidity soon after priming.

Conclusion

Priming UFB water 20 ppm for 120 minutes as a pre-planting treatment can increase the viability of bean seeds, particularly for seeds with low initial viability (germination percentage about 60%), however, the

effect is insignificant for seeds with greater initial viability. The priming treatment boosted seed vigor in all evaluated seed lots.

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