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Determination of Seed Physiological Maturity and Invigoration using Plasma-Activated Water and Ultrafine Bubble Water on Okra Seeds

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Abstract

Seeds physical and physiological qualities are primarily determined by seed physiological maturity. Seed enhancement technology has developed rapidly, including using ultrafine bubbles (UFB) and plasma-activated water (PAW) to increase seed viability. This study aimed to determine seed physiology maturity on okra seed to get the optimal harvesting period seeds and to examine the most effective seed enhancement plasma-activated water and ultrafine-bubble Water. The first experiment was arranged using completely randomized design with fruit maturity as the single factor, harvested at 3, 4, 5, or 6 weeks after anthesis (WAA), with three replications. The second experiment used factorial completely randomized design; the first factor is seed lots with different storage times (fresh seed and one-year storage seed), and the second factor seed invigoration using UFB (20 ppm dissolved oxygen) and PAW (10-, 20-, and 30-min exposure) as the second factor. Untreated seeds were used as control. Fifty seeds were used in each treatment. Okra seed physiological maturity was reached at 5 WAA based on seed dry weight, germination capacity, seedling vigor, and speed of germination. The pod color at 5 WAA is olive brown, and seed testa has dark greyish purple. Plasma-activated water at 10 minutes exposure increased the vigor index of seed lots stored for one year by 86% and PAW20 by 87%. Plasma activated water at 10-, 20- and 30-minutes exposures significantly increased the seedling vigor index and germination speed of fresh seeds harvested at 5 WAA.

Keywords: *Abelmoschus esculentus*, germination capacity, moisture content, seed color, seed dry weight

Introduction

Okra (*Abelmoschus esculentus* L.) is a horticultural species belonging to the family Malvaceae and has been known to the public for quite a long time. This vegetable is distributed in tropical and subtropical regions (Swamy, 2023). Okra fruits can be consumed as a vegetable or medicine because of its high nutritional content. The okra fruits contain 9.6% carbohydrates, 2.25% protein, 1.1% fiber, and 0.2% fat (Khan and Rab, 2019).

Okra plants are generally generatively propagated by seeds. One of the main obstacles to this propagation is the low quality of the harvested seeds; the seeds suitable to be used as planting materials must have optimal physiological maturity. Maximum seed vigor is a benchmark for achieving physiological maturity (Li et al., 2022). Syarovy et al. (2013) stated that seedling growth of rosella seed is strongly influenced by the level of seed maturity, and physiologically mature seeds have maximum storage reserve.

It is important to determine the optimal time of harvest to produce seeds that are physiologically mature (Demir and Ermis, 2005). Seed viability and vigor testing can indicate the physiological quality of the seeds (Bortey and Dzomeku, 2016). Seed dry weight, viability, and vigor are important factors that determine seed maturity. Groot (2022) stated that the dry weight of seeds reaches its maximum when they reach physiological maturity. Bortey and Dzomeku (2016) reported that okra seeds “Asontem” at 10 days after anthesis (DAA) had a maximum dry weight of 2.43 g with a germination percentage of 71%, whereas seeds that were harvested at 40 DAA had a maximum dry weight of 4.10 g and a germination percentage of 73%. Demir and Ermis (2005) showed that okra seeds harvested at 25 DAA had a maximum

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dry weight of 47 mg, with a germination percentage of 47%. When seeds were harvested at 40 DAA, they had a maximum dry weight of 63 mg, with a germination percentage of 76%. Apart from the dry weight of the seed, the maximum germination and seedling vigor are the important physiological maturity criteria. Studies on the harvest time for okra seeds have been reported by Akhir et al. (2017); okra seeds at 38 DAA produced a germination percentage of 70%, whereas those harvested at 42 and 46 DAA had the highest germination percentage at 96%. Another study by Nitish et al. (2021) reported that okra "Super Green" harvested at 25 DAA produced a germination percentage of 76%, whereas seeds harvested at 40 DAA had the highest germination percentage of 91%.

The ultrafine bubble technology (UFB) is defined as gas bubbles with a volume-equivalent diameter of less than 1 μm (Takahashi, 2014). Ultrafine bubble technology can improve seed quality. Because micro bubbles and nano bubbles contained in UFB water can cause the formation of reactive oxygen species (ROS), i.e., free radicals in the form of oxygen, and their derivatives are highly reactive. Liu et al. (2016) reported that ROS application via UFB water can stimulate germination of carrot and spinach seeds. ROS produced by oxygen are hydroxyl radicals (OH). Research by Maia et al. (2021) on sandalwood seeds treated with UFB20 for 24-h increased germination by 4.55%, whereas Iswara (2019) showed that rice seeds treated with UFB20 for 24 h increased the germination percentage by 97.5%. However, treatments with UFB20 and PAW10 on all seed lots were not significantly different.

Plasma is a partially or wholly ionized gas that can be formed in low atmospheric conditions and consists of charged species (electrons and positive and negative ions), neutral species (radical and non-radical atoms and molecules), electric fields, and photons (Scholtz et al., 2015). Plasma treatment can be applied on seeds directly and indirectly. The indirect method does not affect the seeds directly, but instead affecting the gas and water activated by plasma. Plasma-activated water (PAW) increases the content of reactive oxygen and nitrogen species (RONS), and its application during priming can increase seed germination (Nalwa et al., 2017). Water activated by plasma causes changes in biochemical properties. PAW produces acidic conditions that result in the formation of ROS and RONS, thereby creating changes in redox potential and conductivity that can encourage germination and increase seed vigor, root and vegetative growth, and plant reproduction (Al-Sharify et al., 2020). PAW can increase plant growth and development due to an increase in reactive nitrogen species, such as nitrate and nitrite (Bafoil et al., 2019). The positive effect of plasma treatment is sterilizing seeds by reducing the harmful effects of chemicals and pesticides that can harm the environment and eliminating fungal spores on seeds (Khamsen et al., 2016).

Research on UFB water and plasma continues to develop and has excellent potential to be developed to increase seed vigor, including in okra seeds. Information on the physiological maturity of okra seeds was reported by Akhir et al. (2017) and Nitish et al. (2021). However, there is no information regarding the exact physiological maturity of the IPB okra "Naila". Therefore, this study aimed to identify the physiological maturity level of okra "Naila" seeds, and to determine the effects of seed invigoration using PAW and UFB water technology on okra seed quality. Invigoration was conducted on two seed lots, i.e., seeds that have been stored for a long time, and fresh seed lots.

Material and Methods

The research includes okra seed production and seed quality testing. Okra seed production was carried out in two planting seasons, both conducted at the Cikarawang experimental station. The first season was conducted to study seed invigoration from April to June 2022; the second season was conducted to study seed physiology maturity, in January to April 2023. Seed testing was conducted from July 2022 to September 2023 at the Seed Storage and Testing Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture; Biosystems Environmental Engineering Laboratory; F-Technopark, Department of Mechanical and Biosystems Engineering, Faculty of Agricultural Technology, IPB University.

The study used the IPB green okra seeds "Naila", chicken manures, dolomitic lime, NPK Mutiara fertilizer, foliar fertilizer Gandasil, insecticide Curacron, fungicide Antracol. Other materials are 70% alcohol, Royal Horticultural Society color guide to identify the color of okra pods and seeds, UFB generator (FZ1N-10, IDEC), a plasma generator type dielectric barrier discharge, and … to measure dissolved oxygen and ozone.

Experiment 1. Determination of Physiological Maturity of Okra Seeds

The experiment was arranged using a completely randomized design, consisting of four treatment levels: 3, 4, 5, and 6 weeks after anthesis (WAA), three replication. Pods were harvested according to maturity stage starting from 3, 4, 5, and 6 WAA. The physiological maturity of okra seeds in the field is

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determined by marking each flower that blooms using a tagging label. After harvesting, the pods were dried using sun drying for 1-2 days. The seed extraction was carried out manually by peeling the dry pods using hands, and the okra seeds were dried using sun drying for 6-10 days. Seed quality was tested by observing pod color, seed color, seed moisture content, dry weight of seedling, germination percentage, seedling vigor index (SVI), and germination speed.

1. Pod and seed color
   Pod color was identified using the RHS color
2. Seed moisture content
   Seed moisture content is measured at harvest. The moisture content testing procedure follows ISTA (2018), using 5 g seed. The crucible porcelain and lid used as a container are weighed (M1). The seeds, crucible porcelain, and lids were weighed (M2), then placed in an oven at a temperature of (103 ± 2°C) for 17 ± 1 hours. The weight of the seeds after being oven-dried along with the crucible porcelain and lid is weighed again (M3), then the percentage of final seed moisture content is calculated using the following formula:

$$\text{Seed moisture content (\%)} = \frac{\text{M2} - \text{M3}}{\text{M2} - \text{M1}} \text{x } 100\%$$

M1 = weight of crucible porcelain + lid
M2 = weight of crucible porcelain + lid + seeds before oven drying
M3 = weight of crucible porcelain + lid + seeds after oven drying

3. Dry weight of seedling
   Seed dry weight testing was conducted at 103 ± 2°C for 17 ± 1 hour. The formula used is as follows (Perwira et al., 2019):

$$SDW = K_1 - K_0$$

Information:
SDW = seeds dry weight
K0 = weight of crucible porcelain
K1 = weight of crucible porcelain + seeds after oven drying

4. Germination percentage
   The Germination test using the between paper (BP) method, the first observation and measurement were conducted on day 4 and the final observation on day 21 according to ISTA (2018). The germination percentage is calculated using the formula:

$$\text{GP (\%)} = \frac{\text{Number of NS I} + \text{Number of NS II}}{\text{Total seeds germination}} \text{x } 100\%$$

Information:
GP = germination percentage
NS I = normal seedlings on the first count (day 4)
NS II = normal seedlings on the second count (day 21)

5. Seedling vigor index (SVI)
   The percentage of normal seedlings (NS) in the first count of the germination test, the formula used is as follows:

$$\text{SVI (\%)} = \frac{\text{Number of NS count I}}{\text{Total seeds germination}} \text{x } 100\%$$

6. Germination speed
   Germination speed was measured every day until the last observation on day 21 by counting the number of normal seedlings and the difference in hours of each observation (%NS/etmal). The germination of speed calculated using the Sadjad (1994). SG was calculated using the formula:

$$\text{Germination speed} = \sum_{o}^{t} \left(\frac{\%\ NS}{etmal} \right)$$

t = Observation time
% NS = percentage of normal seedlings at each observation time
Etmal = observation time every 24 hours

Experiment 2. Invigoration of okra seeds using Plasma-activated Water and Ultra-fine Bubble Water

The two-factor factorial experiment was conducted using a completely randomized design. The first factor is the seed lot consists of four vigor levels (high, medium and low vigor): the old seed lot harvested at 07/02/2022 at the age of 5 WAA (LM 5) and the lot harvested at 06/25/2022 at the age of 6 WAA (LM 6); both lots were stored for one year at a temperature of 20 ± 1°C and lots harvested on 11-12/05/2023 at the age of 5 and 6 WAA (LB 5 and LB 6) as fresh seed lots. The second factor is seed invigoration, consisting of six levels: control (without invigoration), soaking seed on distilled water, UFBW 20 ppm for 24 h (UFB20), and PAW with an exposure duration of 10 (PAW10), 20 (PAW20), and 30 min (PAW30). The experiment consisted of 24 treatment combinations replicated four times, resulting in 96 experimental units. Each experimental unit was a germination roll containing 50 seeds each using BP method (ISTA, 2018).

Seeds without invigoration were used as controls. The invigoration process was carried out by soaking the seeds for 24 h at a temperature of 20 ± 1°C in

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distilled water, UFBW dissolved oxygen at 20 ppm was injected for 55 min (Maia et al., 2021), and PAW resulted from exposure for 10, 20, and 30 min. PAW measurement results of exposure for 10, 20, and 30 minutes produced a concentration of dissolved oxygen (DO) 32.0-28.8 ppm, ozone 0.05-0.09 ppm, and pH 7.43-7.16. The influence of invigoration is observed by testing germination percentage, vigor index and germination speed.

Data Analysis

Data were analyzed using analysis of variance (ANOVA) using the Duncan Multiple Range Test (DMRT) test at the α = 5% level using SAS 9.4 software.

Result and Discussion

Experiment 1. Determination of Physiological Maturity of Okra Seeds

Description of Okra Flower, Pods and Seeds

Flowering okra begin 48 days after sowing. The trumpet-shaped okra flowers appear on each branch of the okra stem (Figure 1 A) and have five to six yellowish-white petals with a red spot in the center of the flower (Figure 1 B), in one okra plant there are approximately 15-27 flowers per plant. One okra plant produces at least 1-5 flowers every day. The flowers bloom around 06.00 in the morning, pollinate and wither in the afternoon around 17.00. Research by Akhir et al. (2017) reported that the flowers of okra grown in Padang West Sumatra bloomed at 06.00-10.00, like what was reported by Dhankhar and Mishra (2004), that okra grown in India had flowers bloom at 06.00-08.00 and began to wilt at 14.00.

Pods are marked (tagged) using tagging labels with different color variations, aimed at making it easier to obtain seeds at several stages of maturity (Figure 2). The cylindrical pods are 18.5-21 cm, hollow, have pointed tips that are light green, dark green, or brownish if the fruit has reached physiological maturity (Table 1), and contain 20-130 seeds per pod. The shape of okra pods and the seed content inside depends on the size of the pod (Figure 3 A). Okra seeds were oval, 6.03 mm long and 6.42 mm wide. The surface of the seeds was slightly hairy and had lines of small cavities, dark green, which turned brown when dry (Figure 3 B). The development of okra pods and seeds were shown in Tables 1 and 2. Color is one of the essential visual and physical parameters of various fruit species, including vegetable crops, and it can be evaluated automatically using different computer visual systems (Pathare et al., 2013). The color of the okra pods changed as the maturity level increased from the yellow-green group to the gray-green group. The color of the pods at maturity stages 3 and 4 WAA had different color identifications from the top to the base in one pod. At the ripe stage of 3 WAA, the top end to the middle of the okra pods were colored brilliant yellow-green, and then at the base end of the colored pods, they were pale yellow green (Table 1). As for the color of the pods at maturity level 4 WAA, the top end of the pods is intense yellow-green, and then in the middle to the tip of the base of the colored pods, it is light yellow green. The color of the pods was uniform at maturity levels of 5 and 6 MSA. The pods at the maturity level of 5 WAA were colored moderate olive-brown, and those at maturity stage 6 WAA were colored light olive-brown.

Figure 1. Flowers appear on the axillary branch of the okra plant (A) and okra flowers at anthesis (B)

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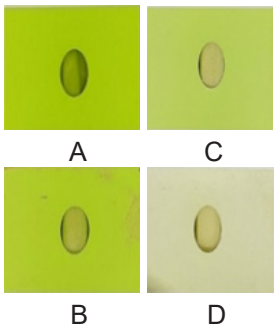
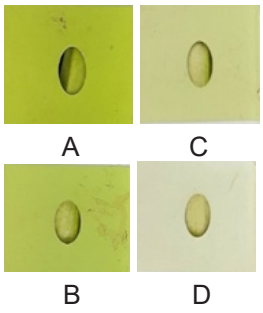
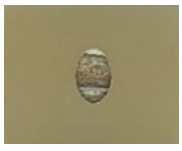
Table 1. The color of okra pods at various level of maturity based on time of harvest after anthesis.

The morphology and color of okra pods				
Maturity Level (WAA)	Color		Code	Picture
3	Yellow-green Group 	Brilliant yellow green (A, B, C), pale yellow-green (D)	RHS2015 -149A, B, C, D	 A C B D
4	Yellow-green Group 	Strong yellow green (A), light yellow green (B, C, D)	RHS2015 -145A, B, C, D	 A C B D
5	Grey-brown Group 	Moderate olive brown	RHS2015 -199A	
6	Grey-brown Group 	Light olive brown	RHS2015 -199B	

Notes: WAA (week after anthesis); RHS (Royal Horticultural Society). 3 WAA *A-C = the top end to the middle of the okra pods, *D = the base end of the colored pods. 4 WAA *A = the top end of the pods, *B-D = the middle to the tip of the base of the colored pods.

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Figure 2. Okra on the plant bed with colored labels indicating different levels of maturity

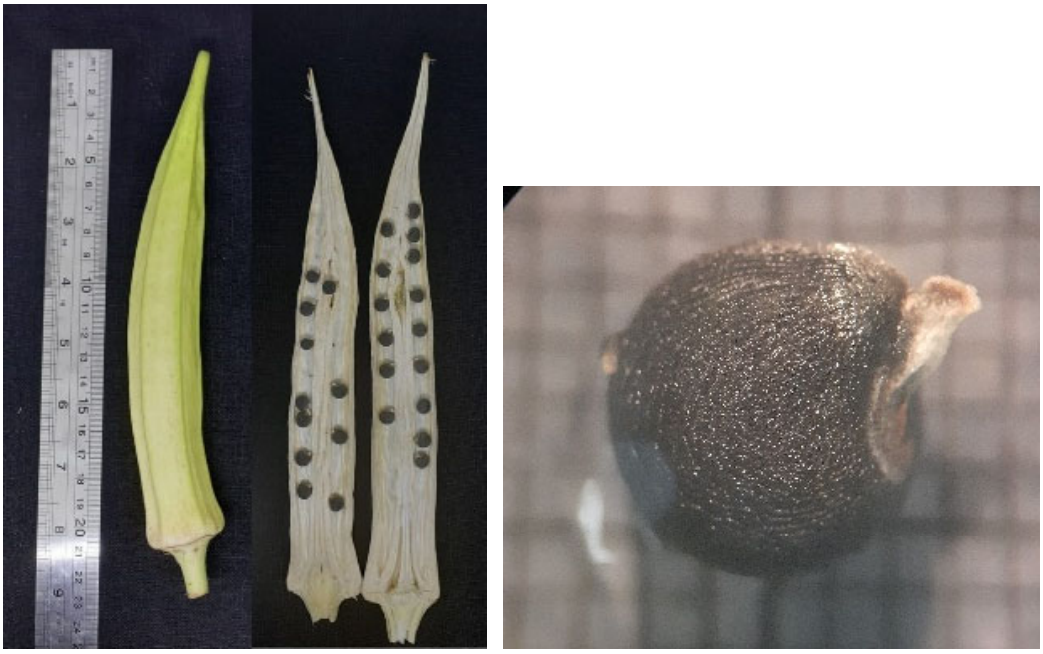

Figure 3. Okra pods and seed arrangements in the pods (A) and an okra seed (B).

Okra seeds at maturity stages 5 and 6 WAA are classified into the same group, viz., the black group (Table 2). At maturity level 3, WAA is classified into the yellow group, while at maturity level 4, WAA is classified into the brown group. The color of the seeds at maturity stage 3 WAA is light yellow; at maturity level 4 WAA is dark greyish reddish brown; at maturity level 5 WAA is dark greyish purple; and at maturity level 6 WAA is black. This is similar to the findings of Bortey and Dzomeku (2016), who showed that okra seeds harvested at the age of 40 and 50 DAA have a black-greyish color. The older the seed harvest age, the darker the seed color. The results of the germination test for light yellow seeds (3 WAA) had low viability (54%), whereas dark-greyish purple (5 WAA) seeds had the highest viability, starting at 4 WAA (80%) and reaching maximum viability at 5 WAA (90.7%) (Table- 2 and 4). This finding is important

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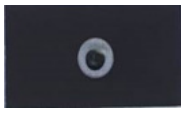
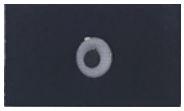
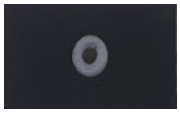
as it shows that seed color at harvest is one of the factors determining the maturity and quality of seeds at physiological maturity.

Fresh seeds have a relatively higher moisture content than physiologically mature seeds. As the level of seed maturity increased, the relative moisture content

Table 2. Okra seed morphology and color at various level of seed maturity based on time to harvest after anthesis

Maturity Level (WAA)		Color	Code	Image
3	Yellow Group 	Light yellow	RHS2015 -10C	
4	Brown Group 	Dark greyish reddish brown	RHS2015 -200A	
5	Black Group 	Dark greyish purple	RHS2015 -202A	
6	Black Group 	Black	RHS2015 -203A	

Notes: WAA=week after anthesis; RHS= Royal Horticultural Society.

Physiological maturity of okra seeds

Okra pods harvested at the maturity level of 3 WAA had the highest moisture content (63.9%), decreasing significantly from 4 to 6 WAA (Table 3). The moisture content at the maturity level of 5 WAA (20.8%) was not significantly different from that at the maturity level of 6 WAA (21.3%). A similar phenomenon occurred with the decrease in seed moisture content during the harvest period at physiological maturity in the study by Demir and Ermis (2005). The maximum dry weight of seeds at a maturity level of 5 WAA was in line with the decrease in the seed moisture content. This was not significantly different from the maturity level at 6 WAA. This shows that the seeds reached physiological maturity at the maturity level of 5 WAA.

The dry weight of seeds reaches a maximum when they reach physiological maturity (Santos et al., 2020). This is also supported by Kartasapoetra (2003) statement that seeds have high viability and vigor when they are physiologically mature, as well as their dry weight. Seeds undergo the histo differentiation stage along with division and cell enlargement. The seed increase phase followed this stage in the form of food reserves. This pattern was indicated by an increase in seed dry weight and a gradual decrease in moisture content.

of the seeds decreased, and the dry weight of the seeds increased.

Seed maturity and development are complex processes that occur over specific periods. The accumulation of food reserves, cessation of embryo growth, tolerance to drying stages, and induction of dormancy are related to seed maturity (Nitish et al., 2021). During the ripening process, the seeds' moisture content varied at each maturity stage. Harvested seeds are still young when they have not yet reached physiological maturity and have higher moisture content. As the maturity of the seeds increased, their moisture content decreased. Nitish et al. (2021) showed that seed moisture content decreased with increasing seed maturity 25 days after anthesis (DAA) at 54.7%, decreasing at 40 DAA, at 18.7%.

The dry weights of the seeds at maturity levels of 5 and 6 WAA were significantly higher than those at maturity levels of 3 and 4 WAA (Table 3). The dry weight of seeds at maturity levels of 5 and 6 WAA reached maximum pod filling (mass maturity). This significant increase in maturity levels at 5 and 6 WAA was related to cell division and enlargement. This is followed by the filling of food reserves into the seeds through the release of water from the cells and the accumulation of material at the location of the food reserves (endosperm) (Santos et al., 2020). The

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Table 3. Seed moisture content and dry weight at several of maturity levels based on time to harvest after anthesis.

Time of harvest (WAA)	Seed moisture content (%)	Dry weight per seed (g)
3	63.9 a	1.81 c
4	57.2 b	2.12 b
5	20.8 c	3.97 a
6	21.3 c	3.95 a

Notes: Values with the same letter in the same column are not significantly different based on DMRT at α = 5 %, WAA=week after anthesis.

maximum dry weight of seeds reflects the maximum supply of food reserves, so the seeds have reached physiological maturity. Bortey and Dzomeku (2016) observed that okra seeds of the Asontem variety harvested at 40 DAA had a maximum dry weight of 4.10 g with a germination percentage of 73.0%.

Sripathy and Groot (2023) stated that mass maturity or physiological maturity is when the seed reaches the highest filling level and the filling period ends, followed by the formation of tissue that closes the vascular tissue pathway between the mother plant and the seed. Seeds are hygroscopic, and this is the beginning of a period of drying or decreasing moisture content when the moisture content is no longer influenced by the parent plant but is influenced by the environment.

The minimum germination standard for okra seed is 75% (Indonesian Ministry of Agriculture, 2019). Table 4 shows that the maturity levels of 4, 5, and 6 WAA had a germination percentage that was not significantly different and was significantly higher than that of 3 WAA. This shows that seeds harvested early before they are physiologically mature produce a low percentage of germination, vigor index, and germination speed. Seeds that have reached physiological maturity have maximum food reserves; thus, seed viability is high (Syarovy et al., 2013). The viability and vigor of okra seeds increase with seed maturity (Bortey and Dzomeku, 2016). Priatama et al. (2022) stated that seeds harvested before physiological maturity can produce low-quality seeds.

The maturity level 5 WAA produced the highest vigor index percentage (39.3%), which was not significantly different from the 4 WAA maturity level but it was significantly different from the 3 and 6 WAA maturity levels. Speed germination at the maturity level of 5 WAA produced the highest percentage (18.4%), significantly different from the treatments at 3, 4, and 6 WAA. The percentage of germination and seed vigor are essential parameters for determining the physiological maturity of seeds, and Akhir et al. (2017) showed that okra seeds at the maturity level of 46 DAA had the highest germination capacity (96%) and vigor index (94%). Nitish et al. (2021) reported that varietal okra seeds are super green; those that developed reached maturity germinated at 25 DAA (76%) and had the highest germination rate at 40 DAA (91%). The low vigor index at maturity levels of 4 WAA (Table 4) shows that seeds not yet physiologically mature can still germinate. However, their vigor is lower, and their germination is weaker than that of physiologically mature seeds (Copeland and Mcdonald, 2012). The results of this study show that harvesting at a maturity level of 5 WAA can produce a high germination percentage, vigor index, and germination speed rate in the Naila IPB okra variety. Therefore, it is best to harvest the seeds at a maturity level of 5 WAA.

Table 4. Viability and vigor of okra seeds with different maturity levels based on time to harvest after anthesis.

Time of harvest (WAA)	Germination (%)	Seedling vigor (%)	Germination speed (%NS/etmal)
3	54.0 b	0.0 c	9.7 c
4	80.0 a	26.7 ab	16.5 b
5	90.7 a	39.3 a	18.4 a
6	86.0 a	24.7 b	16.1 b

Notes: Values with the same letter in the same column are not significantly different based on DMRT at α = 5 %, WAA=week after anthesis.

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Experiment 2. Invigoration of Okra Seeds using Plasma-activated Water and Ultra-Fine Bubble Water

Invigoration with ultra-fine bubble water for 20 minutes (UFB20) increased the germination percentage in all seed lots (Table 5). Invigoration treatments did not increase germination percentage of stored seeds (LM5) and freshly harvested (LM6) seed lots. This shows that small bubble sizes (100-200 nm) can penetrate the seed coat and hydrate the seeds, thereby increasing seed germination (Yabe, 2022; Liu et al., 2014). UFB can enter seeds through the seed coat, and oxygen translocated to the seed increases respiration, thereby enabling energy production for germination (Gomes and Garcia, 2013). UFB water can soften the seed coat by penetrating the cell wall and accelerating water absorption, and ROS produced by UFB can stimulate endogenous germination and oxygen (O_2) in the seed. ROS produced by oxygen are hydroxyl radicals (OH), and the hydroxyl radicals produced by UFB water affect the germination of vegetable seeds (Liu et al., 2016). Liu et al. (2018) reported that the effect of UFB water on carrot and spinach seeds had a positive effect on germination. Research by Maia et al. (2021) on sandalwood seeds treated with UFB20 for 24 h increased normal sprouting by 4.55%, whereas Iswara (2019) showed that rice seeds treated with UFB20 for 24 h increased the germination percentage by 97.5%. In our study, however, the invigoration treatments with UFB20 and PAW10 on all seed lots were not significantly different (Table 5).

Invigoration treatment to LM5 seeds with plasma activated water treatment for 20 minutes exposure (PAW20) produced the highest vigor index and was not significantly different from PAW10 at LM 6 and PAW 20 at LB 5 (Table 5). PAW treatment can

Table 5. Interactions of seed lot and invigoration treatment on okra seed germination percentage, seedling vigor, and speed of germination.

Invigoration treatment	Seed lot			
	LM 5	LM 6	LB 5	LB 6
		Germination (%)		
Control	97.5ABa	97.5ABa	91.0Aa	94.0Aa
Distilled water soaking	99.5Aa	96.5ABa	94.0Aa	96.5Aa
UFB20	98.5ABa	99.5Aa	95.0Aa	94.5Aa
PAW10	96.5Ba	97.5ABa	97.5Aa	92.5Ab
PAW20	99.0ABa	92.5Bb	95.0Aab	85.5Bc
PAW30	99.0ABa	93.0Ba	93.5Aa	82.5Bb
		Seedling vigor (%)		
Control	66.5Ca	64.5Bab	48.0Bc	53.0Abc
Distilled water soaking	43.5Da	43.0Da	28.0Cb	32.0Bb
UFB20	39.5Da	34.0Ea	34.0Ca	33.5Ba
PAW10	72.5BCab	86.0Aa	68.5Ab	47.5Ac
PAW20	87.0Aa	55.5Cb	76.5Aa	33.0Bc
PAW30	79.5Ba	53.0Cc	63.5Ab	25.5Bd
		Germination speed (%NS/etmal)		
Control	22.8ABa	22.6Aa	20.0BCb	21.5Aab
Distilled water soaking	19.5Da	18.8Cab	16.6Cc	17.7BCbc
UFB20	20.2CDa	19.4BCab	18.8Cab	18.6Bb
PAW10	21.3BCbc	23.7Aa	22.6Aab	20.2Ac
PAW20	23.9Aa	20.2Bc	22.5Ab	16.8CDd
PAW30	23.7Aa	20.5Bb	21.5ABb	15.9Dc

Notes: Values with the same capital letters in the same column and with lowercase letters in the same row are not significantly different based on the DMRT at α = 5 %, LM 5 = lot harvested at 07/02/2022 at 5 weeks after anthesis (WAA_, LM 6 = the lot harvested at 06/25/2022 at 6 WAA, LB 5 = seed lot harvested in 11-12/05/2023 at 5 WAA, LB 6 = seed lot harvested in 11-12/05/2023 at 6 WAA, PAW10 = plasma-activated water 10-min exposure, PAW20 = plasma-activated water 20-min exposure, PAW30 = plasma-activated water 30-min exposure, UFB20 = Invigoration with ultra-fine bubble water for 20 minutes.

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increase seed vigor, especially for seeds stored for a long time. Modification of the composition of lipid compounds in the seed coat of Arabidopsis seeds was observed after plasma treatment. Arabidopsis seed coat mutants gl2, gpat5, and Col-0, tested in plasma treatment, can increase seed germination. This shows that in seeds treated with plasma, the structure and composition of lipid compounds change before germination, and metabolism changes after germination (Bafoil et al., 2019). The PAW20 invigoration treatment on LM 5 resulted in the highest percentage (23.9%) of speed of germination and it was not significantly different from the PAW10 and PAW30 invigorations on LM 5 and LM 6; and control on LM 6 (Table 5).

All seed lots had the same initial germination rates. Seed lots stored for one year still had significantly higher vigor than new ones. This is because the seed lot has been stored for a long time under controlled temperature conditions, an RH of approximately 50-60%, and a moisture content of 11%; therefore, it is likely that seeds that have been stored for a long time still have high quality. Newly harvested seed lots tend to have a lower vigor index, primarily at LB 6, allegedly because the maturity level of 6 WAA is a lot that has passed physiological maturity. Another suspicion points to conditions in the field that are attacked by pests such as grasshoppers that always eat okra leaves and diseases such as pathogenic fungal infections Cercospora abelmoschi which is on the underside of the leaf and fruit rot. A prolonged plasma exposure treatment may also harm and reduce the seed quality. This can be seen in the parameters of the vigor index and germination percentage, which reduced the percentage in the new lot at 6 WAA.

Water activated by plasma causes changes in biochemical properties. PAW produces acidic conditions that result in the formation of ROS and RONS, thereby creating changes in redox potential and conductivity that can encourage germination and increase vigor, root and vegetative growth, and plant reproduction (Al-Sharify et al., 2020). ROS and RONS produced by plasma are essential in stimulating germination and increasing seed vigor; the content contained in RONS increases and can inhibit microbial growth (Nalwa et al., 2017). ROS and RONS produced by plasma include O3 and hydroxyl radicals, which play a role in microbial inactivation (Dolezalova and Lukes, 2015). Reactive species produced from plasma are the main factors influencing seed germination and plant growth (Billah et al., 2020). Plasma treatment can expand the pore size of seed coats, thereby increasing ROS uptake and imbibition in seeds, leading to the genetic regulation of seeds (Souza and Marcos-Filho, 2001). Several studies have shown that PAW treatment can stimulate germination, such as mung beans (*Vigna radiata*) with a germination percentage of 99.5% (Zhou et al., 2019), soybeans (*Glycine max*) treated with PAW1 and five germination percentages of 100% (Chiara et al., 2018), and TSS (true shallot seed) (*Allium ascalonicum* L.) seeds can increase germination percentage treated with PAW10 of 91,4%, PAW20 of 91,2%, and PAW30 of 90,08% (Raga, 2023).

Conclusion

The physiological maturity of okra seeds based on seed dry weight, germination percentage, vigor index, and speed of germination is reached at five weeks after anthesis. The color of the ripe stage pods harvested 5 weeks after anthesis was olive brown, and the color of the seeds is dark greyish purple. The invigoration with plasma-activated water for 10 minutes treatment increased the vigor index of seed lots stored for one year (LM 6) by 86%, whereas those treated with plasma activated water for 20 minutes (LM 5) by 87%. Seed treatment with plasma-activated water with the duration of 10 to 30 minutes significantly increased the vigor index and speed germination of fresh seeds harvested at 5 WAA. The research results show that invigoration treatment using PAW technology is effective in increasing seed vigor, especially for seeds that have been stored for one-year, compared to other invigoration treatments.

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