Sains Malaysiana 52(2)(2023): 599-611
http://doi.org/10.17576/jsm-2023-5202-21

Plasma-Assisted Priming: Improved Germination and Seedling Performance of Papaya

(Penyebuan Berbantukan Plasma: Peningkatan Percambahan dan Prestasi Anak Benih Betik)

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Received: 25 July 2022/Accepted: 8 November 2022

ABSTRACT

Papaya is a one of the important tropical fruit crops with a global export of 353 ktonnne reported in 2020. The germination of papaya seed is erratic and often non-uniform. Plasma assisted priming of seed was performed at atmospheric pressure in a parallel plate dielectric barrier discharge system. The germination rate of papaya seeds and the growth performance of the seedling were monitored for several treatment plans combining the atmospheric pressure plasma treatment with soaking of seed in deionized water. An enhanced germination rate of 92% was obtained by the plasma assisted priming, compared to the untreated of 60%. The plasma assisted priming performed by plasma treatment and soaking in an opposite sequence showed different requirement and possibly involved different mechanisms. The treatment time was reduced to only 4 minutes for pre-soaked seeds, otherwise the germination rate increased with the plasma treatment time from 3 to 30 min treatment. The plasma assisted priming approaches were also found enhancing seedling growth performance. The treated seedling grows about two times bigger and the dried mass measured after 30 days was more than 100% compared to that of the untreated and hot water treated seeds.

Keywords: Hot water treatment; papaya germination; papaya seedling growth performance; plasma assisting priming; plasma seed treatment

ABSTRAK

Betik ialah salah satu tanaman buah tropika yang penting dengan eksport global sebanyak 353 ktonnne dilaporkan pada tahun 2020. Percambahan benih betik adalah tidak menentu dan selalunya tidak seragam. Penyebuan dibantu plasma untuk percambahan benih betik dilakukan pada tekanan atmosfera dalam sistem nyahcas dielektrik dengan plat selari. Kadar percambahan benih betik dan prestasi pertumbuhan anak pokok dipantau untuk beberapa pelan yang menggabungkan rawatan plasma tekanan atmosfera dengan merendam benih dalam air ternyahion. Kadar percambahan yang dipertingkatkan sehingga 92% diperoleh oleh penyebuan dibantu plasma, berbanding 60% yang tidak dirawat. Penyebuan dibantu plasma yang dilakukan oleh rawatan plasma dan perendaman dalam susunan yang bertentangan mendedahkan keperluan yang berbeza dan mungkin melibatkan mekanisme yang berlainan. Masa rawatan dikurangkan kepada hanya 4 minit untuk benih pra-rendam, jika tidak, kadar percambahan meningkat dengan masa rawatan plasma daripada 3 hingga 30 minit. Pendekatan penyebuan dibantu plasma didapati juga meningkatkan prestasi pertumbuhan anak pokok. Anak pokok yang dirawat tumbuh lebih kurang dua kali lebih besar dan jisim kering yang diukur selepas 30 hari adalah lebih daripada 100% berbanding anak pokok yang tidak dirawat dan yang dirawat dengan air panas.

Kata kunci: Penyebuan dibantu plasma; percambahan betik; prestasi pertumbuhan anak pokok betik; rawatan air panas; rawatan plasma benih

Introduction

Papaya grows in tropical climates and is one of the essential fruit crops cultivated in Malaysia. Papaya fruits are rich in vitamins and minerals making them a suitable raw material for food and pharmaceutical processing industry (Boshra & Tajul 2013). The unique fruit enzyme from papaya, papain is an important medicinal substance (Priatni et al. 2020). Seed propagation of papaya is a crucial part of the plant's adaptive development. The papaya seed is a dicot, with a hard seed coating the cotyledon, epicotyl, and hypocotyl. The poor viability of papaya seeds would worsen rapidly during storage at high temperature and humidity in the tropical environment. Erratic and non-uniformity of seed germination are challenges to the plant preparation. In practice, harvested papaya seeds often loaded with microorganisms. The thick seed coat in one way, served a protection to the seed, while also hindered water uptake and resists radicle protrusion. Ideal seed germination relies on external factors of temperature, humidity and light exposure (Ellis, Hong & Roberts 1991; Gülsoy & Özkan 2013). Internal factors are the phytohormones governing the seed germination; abscisic acid (ABA), gibberellin acid (GA_3) and ethylene (Vishal & Kumar 2018). The ABA acts as an inhibitor in the seed coat and pulp suppress papaya seed germination (Reyes, Perez & Cuevas 1980). Common method to promote seed germination is by hot water soaking (Agada et al. 2020; Dwivedi et al. 2015). Generally, the germination rate of papaya without priming falls within 40% to 60%. Seed soaking in hot water at about 50 °C was reported to give higher germination rate of 70% to 80% (Deb et al. 2008; Yahiro 1979). Chemical method including a combination of GA_3 and potassium nitrate (KNO_3) on papaya seeds treatment resulted a shorter germination time, 11-14 days at 25 °C and 11-12 days at 35 °C reported for 50% germination (Furutani & Nagao 1987). Nitrate was believed to have an important role in the KNO_3 solution. A combination effect of biochemical including KNO_3, 2-chloroethyl phosphonic acid (CEPA) and GA_3 was also confirmed by another report (Zanotti et al. 2014). The germination rate of papaya seeds has been increased by 125.93% after 30 days with increased starch and lipid mobilization, both attributed to the KNO_3 treatment. To the best of our knowledge, at the time of our manuscript preparation, there are no research on plasma assisting priming for papaya seeds reported in literature.

Non-thermal plasma generated at atmospheric pressure by a parallel plate dielectric barrier discharge system was used here. The gasous plasma could penetrate into the seed coat and deliver reactive species to induce germination. The thermal stress caused by the non-thermal plasma is always below 40 °C (Denes, Manolache & Young 1999). The ambient temperature is maintained ensuring that no physiological damage on the seeds. The active species in the non-thermal plasma include electrons, positive and negative ions, neutral, excited or charged molecules and reactive species mainly refer to both free radicals and reactive oxygen species (ROS). Recombination and deexcitation process produces typically ultraviolet radiations (Butscher et al. 2016; Randeniya & de Groot 2015). The energetic electrons and ultraviolet radiation were responsible for inactivation of fungus and reduction of the microorganisms' load (Zahoranová et al. 2016). The effects of plasma treatment on microorganisms' inactivation on maize seed was reported with a complete inactivation of native microbiota after a treatment time of 1 min for bacteria and 3 min for filamentous fungi (Henselová et al. 2012). The high-energy particles in the plasma could induce changes of the seed's surface morphology (Li et al. 2017). The effects of plasma treatment on seeds have been widely investigated in crop seeds like wheat (Meng et al., 2017), maize (Henselová et al. 2012; Zahoranová et al. 2018), radish sprout (Attri et al. 2021), soybeans (Ling et al. 2014), and tomato (Zhou et al. 2011). In general, the water absorption efficiency of the seeds is an important factor. A treated cotton seeds with a lower contact angle of the seed surface was reported to have water absorption efficiency enhanced by 133%. This was achieved by a plasma treatment under a streamer discharge generated by a dielectric barrier discharge at $38_{p\text{-}p}$ kV and 1 kHz for a duration of 27 min (de Groot et al. 2018). The opposite has been confirmed by an experiment using a hydrophobic thin film coated seed, that delayed germination was reported as the result of reduced water uptake (Volin et al. 2000). Wheat seeds treated by an air plasma generated at 13 kV and 50 Hz reported with better water absorption was due to plasma etching effect on the seed coat (Zahoranová et al. 2016). The treated seeds gave an increased germination rate of 9.1% with 7 min plasma treatment. The present work aimed at exploring the effect of plasma assisted priming to maximize the positive effect of plasma enhanced seed germination and to observe the potentially extended effect on the seedling growth performance.

Materials and Methods

Plasma Treatment Plan and Papaya Seed Preparation

Papaya seeds (Carica papaya cv. Sekaki, extracted from the mature fruit and dried at room temperature after harvest) purchased from a Kuala Lumpur market were washed to remove the pulp from the surface of the seeds and air dried. The mean moisture content of wash seed was 48.0% and dry seed was 6.8%. Hot water soaking used deionized water heated to 50 °C.

Plasma assisted priming of papaya seeds was conducted with direct plasma irradiation in combination of soaking of seeds. Two methods were performed; first, plasma treatment was applied on the seed samples followed by soaking and second in the reverse sequence whereby the soaked seeds were subjected to plasma treatment. The soaking of the seeds was performed by using deionized water (DI) at room temperature at 29 °C. Three groups of control or reference seed samples were prepared; first group being the dry seeds, misted with deionized water without soaking, second group with soaking only without plasma treatment and a third group with soaking in hot water at 50 °C. The latter two groups of samples were soaked for 48 hours. Each group of the papaya seed samples consist of 50 seeds, and they were labeled according to plasma treatment procedure and treatment time. The overall treatment plan is presented in Figure 1.

The plasma treatment was conducted by using the parallel plates dielectric barrier discharge system at 50 Hz and $32_{p\text{-}p}$ kV, with filamentary discharge observed with a gap distance of 5 mm to 10 mm. The seed samples were distributed uniformly in the gap for direction plasma irradiation. The plasma appeared diffusive in the presence of the seeds. Plasma treatment duration was varied according to the treatment plan.

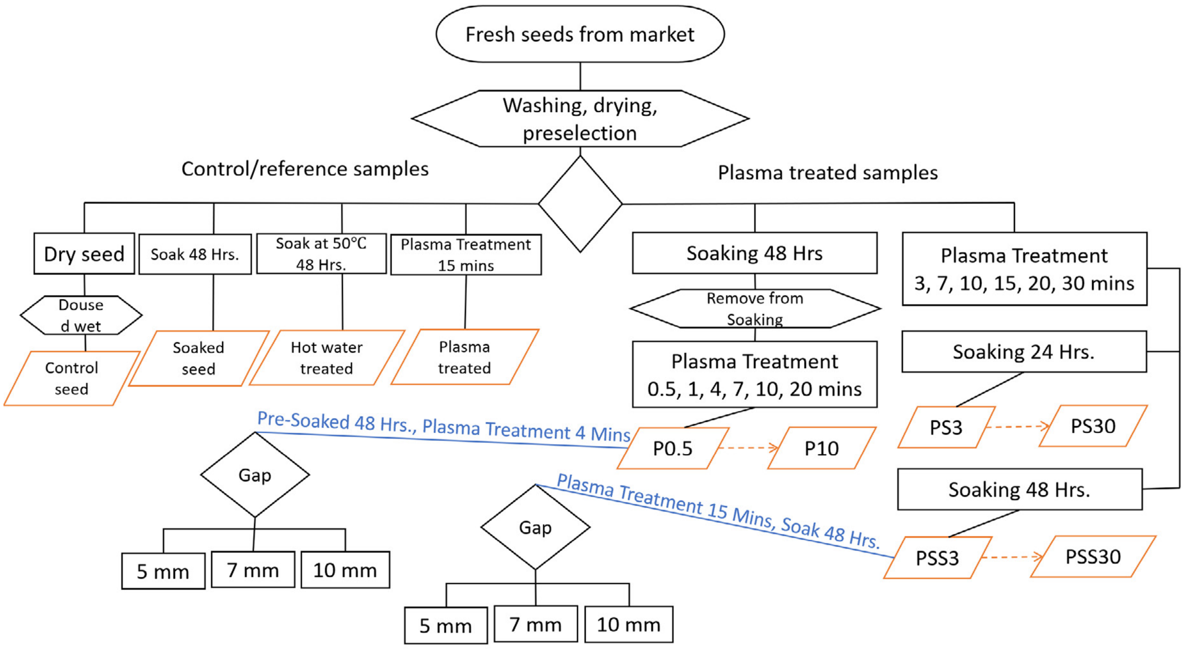

FIGURE 1. Treatment plan for plasma assisted priming of papaya seeds

EXPERIMENTAL SETUP AND DIAGNOSTICS

The parallel plates dielectric barrier discharge (DBD) system consisted of two glass layers as the dielectric barrier covering both electrodes. A groove was made on the glass layer such that the copper electrodes was fitted in with no exposed edge. The electrodes were powered by a 50 Hz high voltage supply. A filamentary discharge was observed in the discharge gap between the glass layers when high voltage was applied. Papaya seed samples were placed in the discharge gap for plasma treatment.

The DBD discharge was measured by using a high voltage probe and a current probe for the real time current and voltage waveforms. The waveforms changed during seed treatment under different discharge parameters.

The measurements were analyzed to plot the respective Lisajour figures. The emission from the plasma was measured by an optical emission spectroscopy using the Ocean Optic Spectrometer HR4000 with the detector directed to the discharge region. Figure 2 shows the arrangement of the experimental setup and the measurement techniques. Emission spectra acquired during a discharge and during seed treatment were obtained.

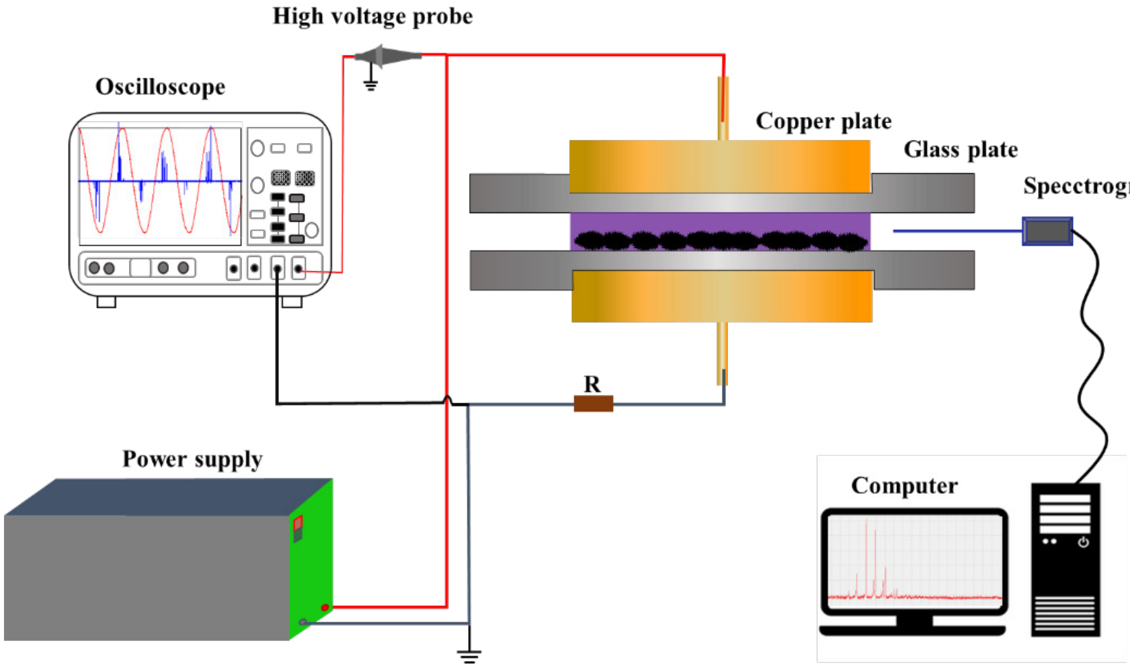

FIGURE 2. The experimental setup and the arrangement of electrical characterization

ANALYSIS OF THE RESIDUE ON SOAKED SEEDS

Papaya seeds were collected after soaking and washed with 30 mL of deionized water (DI) in a container. The solution after seed-wash was reserved in the container that the residue from the soak seeds could be analyzed. It was found to contain a generous amount of NO_2^-, NO_3^-, NH_4^+, and H_2O_2 originated from the surface of the seeds, that could have been generated by the seeds. The reactive species in the as-obtained solution were quantitatively determined using the chemical reaction techniques. The ultraviolet spectrophotometer (Genesys 10S UV-VIS) was used to detect the absorption spectrum of the samples. H_2O_2 concentrations were quantified using the colorimetric method of titanium oxysulfate (TiOSO4, Sigma-Aldrich, product number 89532). A total of 1 mL $TiOSO_4$ solution was added to 5 mL of the as-obtained solution. After 10 min incubation, absorbance was read on a spectrophotometric plate reader at 405 nm wavelength. A standard curve of known H_2O_2 concentrations (0, 0.1, 0.2, 0.3, 0.5, 0.7, 1, 1.5 mM) was included on each plate. The absorbance of the samples was converted into H_2O_2 concentration according to the standard curve. The NO_2^- concentrations was determined by using the one level blue micro spoon nitrite test reagent (Spectroquant, 1.14776.001). The absorbance was read at 545 nm, with reference to $NaNO_2$ solutions of known concentrations (0, 2, 5, 10, 15, 20, 25, 32.5 µM). NO_3^- concentrations were determined photometrically by nitrate test reagent (Spectroquant, 1.09713.001) and calculated using a $NaNO_3$ standard curve, which comprised a set of standard concentrations of 0, 6, 23, 59, 118, 235, 588,1176 µM. For measuring NH_4^+, a total of 0.2 mL Nesler's reagent (Sigma-Aldrich, product number 72190) was added to 5 mL of the solution. After 10 min incubation, absorbance was read on a spectrophotometric plate reader at wavelength of 425 nm. A standard curve of known NH_4Cl concentrations

(0, 14.28, 28.56, 42.84, 57.12, 71.4, 114.24, 142.8 μM) was included on each plate to determine the NH_4^+ concentration. The measurements of particles, pH, electrical conductivity (EC), and temperature of the NO_2^- concentrations were conducted immediately after the collection the as-obtained solutions.

Results and Discussion

Measurements of the Non-Thermal Plasma

The discharge in filamentary mode was characterized by the voltage and current waveforms with multiple current spikes. A typical signal obtained during papaya seed treatment at $32_{p\text{-}p}$ kV is shown in Figure 3(a). The discharge appeared bright with the emission in ultraviolet (Figure 3(b)). The spectra measured consisted of lines correlated to the N_2 second positive system (C-B) at 329, 348, and 371 nm and first negative system of N_2^+ (B-X) at 428 nm (Figure 3(c)). The emission was due to electron impact excitation of molecular ground state of N_2 and N_2^+ in air. This spectrum is often observed in air or nitrogen DBD discharges (Liu, Niu & Yu 2011). Direct charge measurement was obtained by using a monitor capacitor of 0.22 μF. The voltage and charge signals were obtained for the discharge with and without seeds and plotted in the QV Lissajous figures shown in Figure 3(d). The dissipated power of the DBD was calculated according to Equation (1),

$$\mathrm{P} = f \cdot \mathrm{E} = f \cdot \oint_{\mathrm{T}} \mathrm{U(t)} \frac{dQ}{dt} dt \tag{1}$$

where P denotes the power and f represents the frequency. The dissipated power obtained from the Lissajous figures for discharge alone was 8 mW, for dry and wet seed treatments were 75 mW and 100 mW. The equivalent dielectric constant of the gap increased with papaya seeds resulting in an increase of capacitance and charge. Higher power was measured with wet seed treatment.

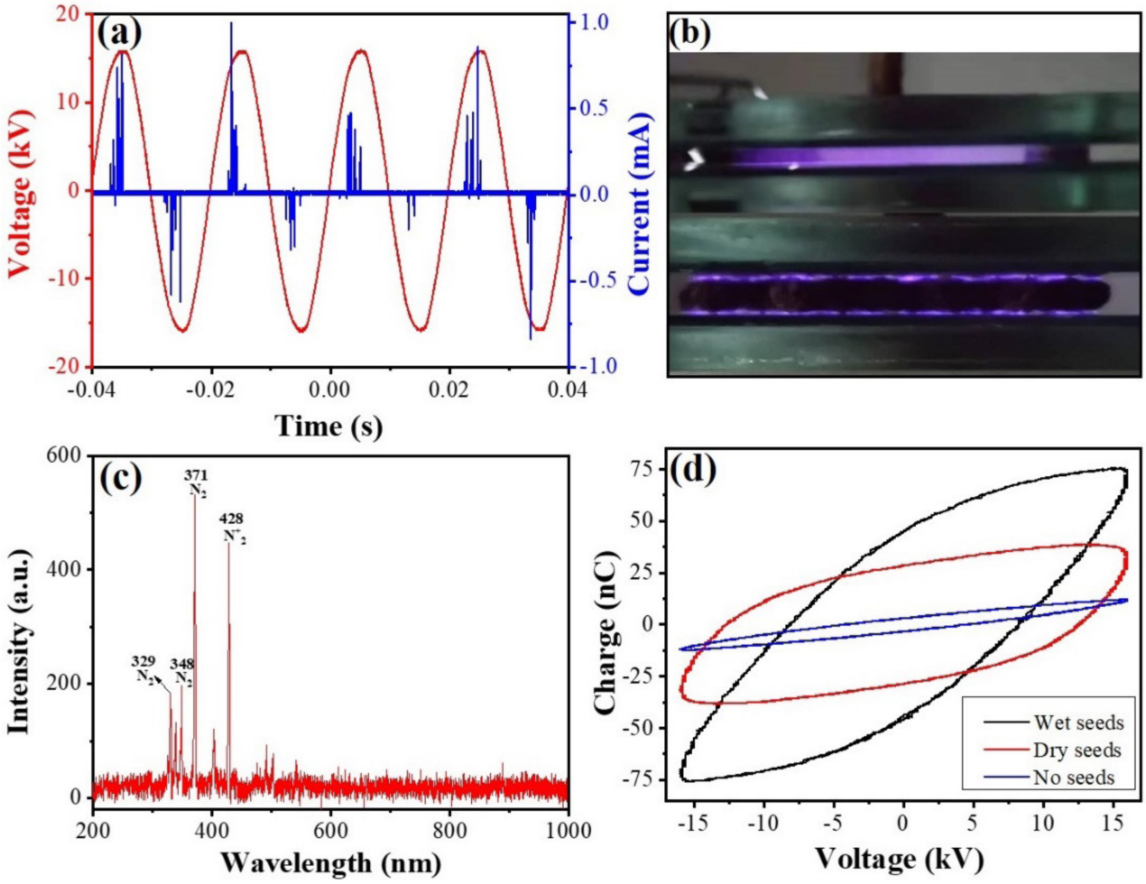

FIGURE 3. (a) The voltage and current waveforms, (b) photographs of discharge gaps without and with seed sample, (c) emission spectrum and (d) and Lissajous figures

PAPAYA SEED GERMINATION WITH PLASMA TREATMENT

The effect of plasma treatment was investigated experimentally by observing the germination rate of the treated papaya seeds. Four groups of sample were observed in the germination experiment; a control group using papaya seeds placed on a filter paper doused with deionized water without plasma treatment, second group of papaya seeds were treated in the DBD discharge for 15 min, third and fourth groups were also treated 15 min; whereby group 3's treated seeds were soaked in 20 mL of deionized water for 24 h and the group 4's treated seeds were soaked for 48 h. The four groups of seeds were prepared on the germination plates and placed in a greenhouse with a temperature of 30 °C. The germination data was taken daily at noon from the 4th day to the 14th day. The results obtained suggested that plasma treatment in combination of soaking gave better effect, as shown in Figure 4. The group of seeds treated for 15 min followed by 48-hour soaking demonstrated the highest germination rate and shortest germination time. A recent paper reported that plasma treated papaya seeds achieved higher germination rate of 54% compared to 38% for the control samples (Ahmed et al. 2022). In this work we optimized the effect of plasma treatment by combining soaking of seeds.

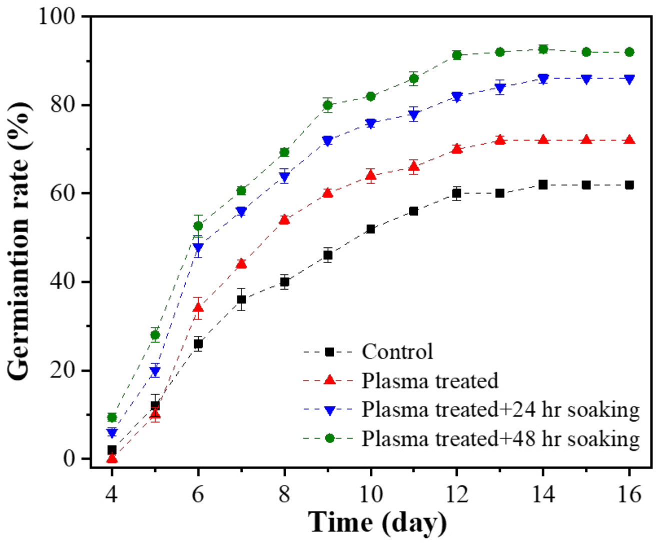

FIGURE 4. Papaya germination rate obtained in plasma treated seeds with and without soaking

OPTIMIZATION OF DISCHARGE PARAMETER FOR SEED TREATMENT

Plasma treatment of the seeds conducted with the parallel plate dielectric barrier discharge system were checked with variation in the gap distances of 5 mm, 7 mm, and 10 mm. Seed treatment time was believed to be the main governing factor towards the best result. The treatment time was varied from 3 min to 30 min and the treated seeds were soaked for 48 hours. Another treatment scheme reversed the sequence; that the seeds were first soaked for 48 hours and received the plasma treatment after that. The plasma treatment time was conducted from 30 s to 20 min. The results obtained for both treatment schemes are plotted in Figure 5. The data points on the graph represent the average of three replicates and the error bars indicating their standard deviations. The group of samples corresponded to the plasma treatment of 3 min plus soaking showed that the germination rate increased linearly from day 4 to day 10 and reached the maximum on day 11, recorded a germination rate of 72%. The seed samples treated for 15 min, 20 min, and 30 min reached the maximum germination rate of 92% at day 13. On the other hand, papaya seeds soaked before plasma treatment and treated for at 4 min achieved 92% germination rate. However, extended treatment time of 7 to 20 min did

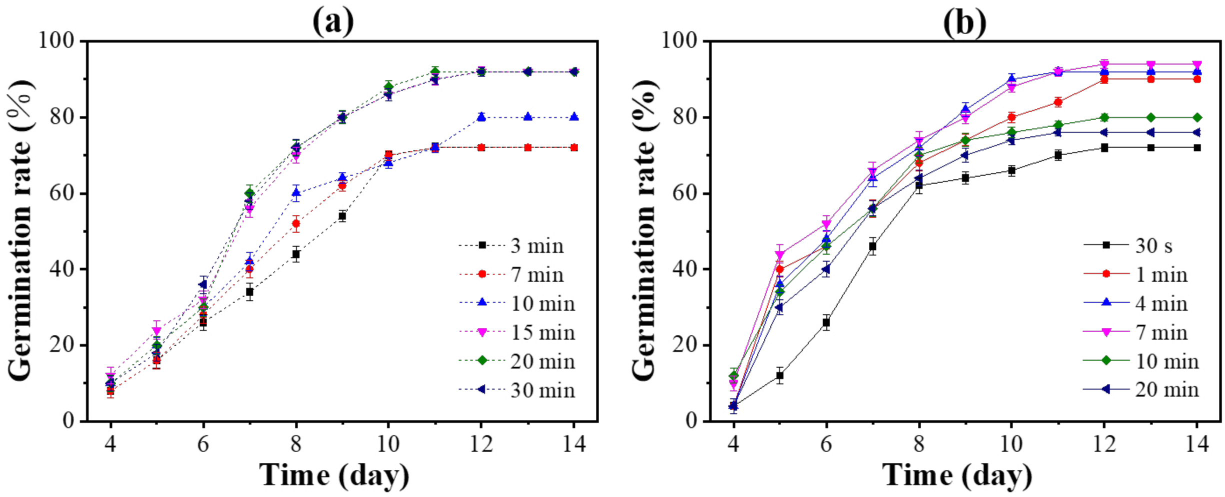

FIGURE 5. The germination rate of papaya seeds (a) plasma treatment for 3 min to 30 min followed by 48 h soaking and (b) plasma treatment on pre-soaked seeds

not improve the germination results but the data shown a saturation at below 75%. Therefore, the two optimum plasma assisted priming schemes were determined as 15 min plasma treatment followed by 48 hours soaking and 4 min plasma treatment on pre-soaked seeds.

EFFECT OF ELECTRIC FIELD ON SEED TREATMENT

The gap distance in the DBD system can be adjusted up to 10 mm while maintaining the same applied voltage of 32 kV_{pp}. The larger gap eased the placement of seeds, while smaller gap produced higher electric field across the samples. The effect of electric field applied on seed treatment was related to the gap distance. By setting the gap distance to 5 mm, 7 mm and 10 min, the papaya seeds were treated for germination observation. The effect of the electric field at different gap distances was investigated for the two optimum parameters for priming; 15 min plasma treatment followed by soaking and pre-soaked seeds treated for 4 min. The treated seeds were germinated for two weeks. The results are summarized in Table 1. The treatment was observed to be less effective and lower germination rate in bigger gap distance. The best result was 92% germination rate on day 13^{th} with 5-mm gap distance.

The germination data for the samples treated with plasma for 4 min after soaking was best the gap distance of 7 mm. The germination rate was 92% recorded on the 13^{th} day. The suitable gap for wet seed treatment was determined at 7 mm.

SUMMARY OF GERMINATION PERFORMANCE

The two optimized procedures for plasma assisted priming were plasma treatment at $32_{p\text{-}p}$ kV, 5 mm gap, 15 min, followed by soaking of seeds and plasma treatment for 4 min at $32_{p\text{-}p}$ kV, 7 mm gap distance on pre-soaked seeds. These two sets of data are compared against the control group that the papaya seeds were untreated and the reference group that the papaya seeds were treated with hot water. The results gave a summary of the efficacy of the plasma assisted priming of papaya seeds, as plotted in Figure 6. The germination rate of the control group increased slowly from day 4 to day 10 day and reached to the maximum on day 12, with a germination rate of 72%. The group of hot water treated seeds achieved a higher germination rate of 80%, with the germination potential remain similar to the control group at about 60%. Both approaches that we have taken to achieve plasma assisted priming of papaya seeds have performed superiorly, giving a higher germination rate of 92% and germination potential of 70% and 76%. The enhancement of germination rate was around 20%.

SURFACE ANALYSIS OF TREATED PAPAYA SEED

The surface tension of the papaya seeds after treatment was obtained by contact angle measurement. The contact angle experiment was conducted in an optical contact angle meter (OCA200). A drop of less than 10 µL make a contact on the seed surface, allowed the contact angle and surface tension to be obtained from the instrument.

TABLE 1. Papaya germination with plasma treatment preformed with different discharge gap distances

A. Plasma treatment at $32_{p\text{-}p}$ kV, 15 min followed by soaking

Time (day)	5 mm	7 mm	10 mm
4	9.3±0.9	8.7±0.9	4.7±0.9
5	28±2	31.3±0.9	22±0
6	53±2	46±2	35.3±0.9
7	60. 7±0.9	54±2	44±0
8	69.3±0.9	68.7±0.9	52±2
9	80±2	72.7±0.9	58.7±0.9
10	82±0	76±2	65.3±0.9
11	86±2	80.7±0.9	69.3±0.9
12	91.3±0.9	82±0	72±2
13	92±0	85.3±0.9	76.3±0.9
14	92.7±0.9	86.7±0.9	76.3±0.9

B. Plasma treatment at $32_{p\text{-}p}$ kV, 4 mins for pre-soaked seeds

Time (day)	5 mm	7 mm	10 mm
4	6.7±0.9	7.3±0.9	0.7±0.9
5	26±2	38±2	16.3±0.9
6	38±0	52.7±2	34.3±0.9
7	46.3±0.9	70±2	56±2
8	62.7±0.9	76.3±0.9	60±2
9	66.3±0.9	84±2	70.3±0.9
10	70±2	86±0	74.7±0.9
11	72±0	86.7±0.9	76±0
12	77.7±0.9	90.3±0.9	82±2
13	80.7±0.9	92±0	84±0
14	80±2	92.3±0.9	84.7±0.9

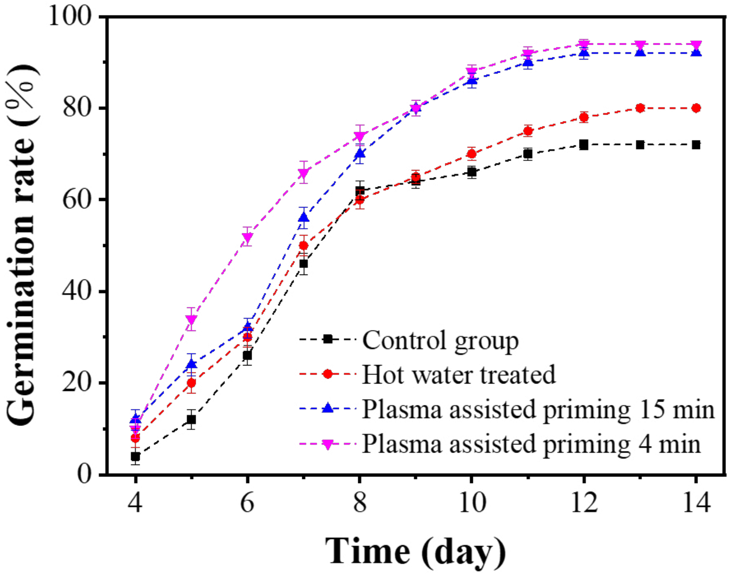

FIGURE 6. Germination rate with plasma assisted priming (15 min + soaking and soaking + 4 min) compared to control and hot water treated samples

This is a direct indication of the surface quality related to water absorption by the seeds. The results of the contact angle measurement are summarized in Table 2, with the contact angle decreased with treatment time. A significant drop in contact angle from 80° in untreated seed to about 40° was achieved after 15 min of plasma treatment. The surface extension decreased to half after 7-min treatment and to below 20 mN/m after 30-min treatment. The surface modification was due to some high-energy particles in the plasma that have caused a physical etching of the seed coat similar to that observed in rice and sunflower seeds (Srisonphan 2018; Thirumdas et al. 2017). In the current experiment, the presence of RONS and UV-photons could have also reacted with the surface of the papaya seed coat and introduce hydrophilic groups, resulting in a chemical modification, such as those reported elsewhere (de Groot et al. 2018; Wannicke et al. 2021). The physical etching and chemical modification enhance the seed absorption of water as a supportive mechanism to break seed dormancy and stimulate germination. The seed coat had become more hydrophilic after treatment and more conducive to water uptake.

TABLE 2. The contact angle and surface tension of papaya seeds

Treatment Time (min)	0	3	7	10	15	20	30
Contact angle (degree)	82	74	65	59	55	54	47
Surface tension (mN/m)	60.27	45.97	28.37	24.88	22.34	20.17	15.56

PARTICLES CONCENTRATION FROM SOAKED SEEDS AFTER PLASMA TREATMENT

The soaked papaya seeds have enhanced germination with 4 min of plasma treatment but not longer. The results indicated that longer treatment time has caused negative effect on the seed germination. It is believed that some reactions have taken place when the seeds were removed from soaking, and plasma treatment on the wet seeds have introduced more reactions. This information has been collected by washing the treated seeds with 30 mL of DI, for all the samples after plasma treatment of 30 s to 20 min. The solution collected contained a generous amount of ion of NO_2^-, NO_3^-, NH_4^+, and H_2O_2 where their concentrations were measured. The acidity measured in pH for the solutions were 3.0±0.2 of 30 s, 2.8±0.2 of 1 min, 2.6±0.2 of 4 min, 2.6±0.1 of 7 min, 2.3±0.2 of 10 min, and 2.1±0.2 of 20 min. The concentrations of the others ionic particles are listed in Table 3, calculated according to the standard calibration curves. The solution contained 32 μM of NO_2^- and 278 μM of NO_3^- particles for the 4-min plasma treatment group has rapidly increased to 51 μM and 461 μM in the 10-min plasma treatment group. H_2O_2 and NH_4^+ in the solution was below the detectable concentration. The acidic condition and nitrite presence in the soaked seeds under plasma treatment at low level could contribute to both microbial inactivation and promote seed germination (Thirumdas et al. 2017), while nitrate is an effective chemical breaking dormancy and promotes seed germination (Duermeyer et al. 2018; Luna & Moreno 2009). However, the present data showed that there was a threshold possibly due to the pH for a positive effect.

TABLE 3. Concentration of particles in residue of soaked seeds

Plasma treatment time	NO_2^- (μM)	NO_3^- (μM)	H_2O_2 (mM)	NH_4^+ (μM)
30 s	10.6335±3.2361	14.6432±3.8347	< 0.1	< 14.28
1 min	20.5613±3.0827	80.018±7.0140	< 0.1	< 14.28
4 min	32.1472±3.4126	277.7269±6.462	< 0.1	< 14.28
7 min	39.4720±2.0133	420.0447±5.6279	< 0.1	< 14.28
10 min	50.6614±2.3362	460.5135±6.6381	< 0.1	< 14.28
20 min	49.5462±2.5981	492.1415±7.4291	< 0.1	< 14.28

GROWTH PERFORMANCE OF THE PAPAYA SEEDLINGS

Papaya seed priming assisted by plasma treatment was conducted to potentially promote rapid and more uniform seed germination, which might also promote germination quality, improve plant growth and disease resistance as well as fruit yield. Plasma-assisted priming approaches presented in the paper have demonstrated enhancement of germination rate. The germinated seeds were sow and grown for 30 days for observation of their growth performance. The plant height was measured for 30 days, and dry mass and root length sum were obtained after the 30 days' observation period. The root length refers to the sum of the papaya root including all the root branches. The growth performance of hot water-soaked seedling did not vary from the control group, indicating the techniques merely stress the seeds to break dormancy without further affecting the quality of the plants. The growth performance of the seedlings developed from the plasma treated seeds was significantly better than the control and hot water-soaked groups. The plant height registered for the plasma-treated seedlings was found increased by 60% to 70%. At the end of 30 days, the average plant height for the plasma-treated seedlings was about 73 to 75 mm, compared to untreated seedling at about 60 mm. The data obtained based on the selected four groups of seedlings are listed in Table 4. Plasma assisting priming has promoted germination and positively affected the early developmental stage of the seedling. This warrants more works to show the mechanism of the plasma treatment on the epigenetics and molecular biology of the seed. Moreover, the papaya seedlings were observed to have developed better root structures after plasma assisted priming. The plasma treated samples has their root length increased by 65 to 70%. Roots served an important function in plant development to ensure essential nutrients and minerals uptake. A strong rooting can also be related to fruit production in their later stage (Wang et al. 2016). The dry mass was the final results obtained concluding the growth of the seedling. The plasma treated samples has the dry mass increased by 44% and 55% for the groups of plasma treated followed by soaking and plasma treatment on pre-soak seeds.

TABLE 4. Growth performance: Plant height, root length and dry mass

A: Plant height (mm)				
Time (day)	Control	Hot water	BS	AS
2	6.5±0.4	7.41±0.4	11±1	11±1
5	21±2	20±2	26±2	26±2
10	29±1	31±1	36±1	37±2
15	36±1	40±1	45±2	45±1
20	45±	50±1	53±2	53±1
25	57±1	60±1	63±1	65±2
30	61±1	62±1	73±1	75±1
B: Root length sum and dry mass after 30 days				
Root length sum (cm)	54±6	68±6	90±7	92±8
Dry mass (g)	0.14±0.01	0.17±0.01	0.20±0.02	0.22±0.01

CONCLUSIONS

The plasma assisted priming of papaya seeds was introduced by combining plasma treatment under DBD and soaking of seeds. Two approaches have been identified as optimum resulted enhanced germination rate as well as better performance in seedling development. Papaya seeds germination without treatment have a germination rate of 72%. Hot water treatment that has always been practiced in local farms was found to give better germination rate of 80%. The germination potential of the two groups were the same, at 60%. The germination rates of plasma assisted priming using $32_{p\text{-}p}$ kV, 5 mm gap, 15 min in DBD treatment followed by soaking and 32p-p kV, 7 mm gap, and 4 min in DBD treatment on pre-soaked seeds are both 92% and their germination potential are 70% and 76%. The early growth performance of the papaya seedling has clearly differentiated the control and hot water treated group with plasma assisted primed group. The plasma treated seedling have growth better achieved more in plant height in the 30 days' observation period, as well as developed better root structures. The overall dry mass obtained after 30 days for the two plasma treated group have increased by 44% and 55% for the plasma treated followed by soaking method and plasma treatment on pre-soaked method. The corresponding mechanism involved on the seed coat was shown by the surface analysis showing a decrease in its contact angle and the surface tension. The saturating mechanisms when plasma treatment was applied on pre-soaked seed was correlated to the biochemical activities related to the concentration of nitrite and nitrate. Plasma treatment has been widely reported as beneficial to inducing seed germination, whereby it can give multitude of effects by reacting on the seed surface as well as via a liquid interface. This emphasizes a potential far-reaching epigenetic impact on the plants, that shall be clarified by more researches and experiments.

ACKNOWLEDGEMENTS

The work is supported by the Fundamental Research Grant FRGS/1/2018/STG02/ UM/02/8 from the Ministry of Higher Education, Malaysia. We acknowledged the financial support from University of Malaya Impact Oriented Interdisciplinary Research Grant (IIRG006A-19FNW).

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