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Effect of Corona Discharge Plasma Radiation on the Viability of True Shallot Seeds (*Allium cepa* L. var. *aggregatum*)

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ABSTRACT

TSS (True Shallot Seed) need to be developed to address the quality and quality of shallot seeds. The seeds, however, still has constraints on its viability and germination. The objective of this study was to investigate the effect of corona discharge plasma radiation on the viability of true shallot seeds. The research was conducted in March - April 2023 at the Plasma Laboratory, Faculty of Science and Mathematics, Diponegoro University, and the germination test experiments at the the Agricultural Technology Research and Assessment Installation (IP2TP Ungaran). The experiment was designed completely randomized with 6 treatments of radiation time consisting of P0 (without radiation), P1 (5 min), P2 (10 min), P3 (15 min), P4 (20 min), and P5 (25 min). All treatments were carried out with 5 replications. The collected data were processed using ANOVA and then continued with the DMRT test. The results showed that the corona plasma radiation treatment for 15-25 min affected the parameters of germination, germination rate, seed growth rate, vigor index, seed uniformity, and sprout length.

1. INTRODUCTION

The productivity of shallots in Indonesia is still relatively low, at 9.92 tons/ha, whereas its potential can reach 12-17 tons/ha (BPS, 2020; Sholikin & Haryono, 2019; Mardiyanto *et al*., 2017; Nurjanani *et al*., 2019). Generally, shallot cultivation still relies on bulbs for propagation. Bulbs used as seeds are obtained from harvested shallots, usually around 40% of the total yield (Sari & Inayah, 2020; Sakti *et al*., 2017; Andalasari *et al*., 2017). However, bulb seeds have some disadvantages, such as a limited storage period of only 3 months. Additionally, continuous vegetative propagation over a long period can lead to inherited seed diseases, resulting in decreased seed quality and narrow genetic diversity (Alfariatna *et al*., 2018; Karim *et al*., 2019).

True Shallot Seed (TSS) is an alternative planting material replacing bulb seeds in shallot cultivation. The advantage of TSS is that it can increase shallot bulb yield up to twice as much as using bulb seeds (production of 26 ton/ha). Moreover, TSS is cheaper than bulb seeds, disease and virus-free, and has a longer shelf life of around 1-2 years. As for the TSS seed requirement, it is also lower, ranging from 2-3 kg/ha compared to bulb seeds (± 1-1.2 tons/ha) (Firmansyah *et al*., 2021).

One potential alternative technology to address the scarcity of shallot seedlings in Indonesia is the use of True Shallot Seed (TSS) (Firmansyah *et al*., 2021). Despite its many advantages, the use of TSS seeds as a substitute planting material for bulb seeds still faces challenges in its viability, thus efforts are needed to accelerate shallot germination and growth in the seedling phase to shorten the time for transplanting to the field. TSS requires between 28-35 days after sowing for the germination phase (Triharyanto *et al*., 2018; Sopha *et al*., 2015).

Efforts to improve the viability of TSS seeds can be done by utilizing plasma technology to accelerate germination and growth. In the world of Physics, there are three types of plasma: thermal plasma, hot plasma, and the one often combined with biology and agriculture is cold plasma. Cold Plasma/Non-thermal Plasma/Atmospheric Cold Plasma (CAP) has been widely applied in the field of biology and agriculture as a seed germination stimulus and food preservation method. Plasma is generated by separating electrons from molecules (ionization) due to high voltage between two electrodes. Corona discharge appears as light in a localized space around the tip of the electrode (Zhou *et al*., 2016). The radiated plasma glow is also called Cold Atmospheric-Pressure Plasma (CAPP), which has a gas temperature of about 37°C and an energy of several electron-volts (1 electron-volt = 11,600 K) (Volkov *et al*., 2019). Through plasma irradiation technology in open air, there is great potential to produce N+ ions. The high nitrogen composition in free air reaches 80% and causes plasma irradiation to have great potential to produce N+ ions.

According to research by Attri *et al*., (2020), on various seeds such as radish, chili, wheat, sunflower, soybeans, garlic, tomatoes, pumpkins, cucumbers, peppers, spinach, basil, and oats, an increase in germination percentage and growth parameters was observed. In mangrove plants, the use of plasma light radiation successfully shortened seed dormancy by 2.4 months (Nur *et al*., 2013).

Plasma light radiation is formed from several types of atoms or molecules originating from the air by using electrodes through a plasma reactor, which can fix nitrogen from the air by breaking down N_2 and O_2 molecules in the air into N and O atoms forming nitrogen oxides (nitric acid or ammonia). In its application, plasma radiation directed at the surface of garlic seed bulbs causes nitrate ions (NO_3^-) produced to accumulate on the surface of the bulbs, thus increasing hydrophilicity and water permeability (Bormashenko *et al*., 2015). Nitrate ions (NO_3^-) or NH_4^+ are absorbed through active transport with the help of energy from NADPH to initiate sprouting (Mukaromah *et al*., 2013).

Nitrogen is the most common element found in essential plant compounds such as proteins, nucleic acids, Deoxyribo Nucleo Acid (DNA), and many vitamin contents. Besides, nitrogen also plays a role in most biochemical reactions that constitute plant life. In plants, nitrogen can be obtained through electrical discharge such as lightning in the form of nitrogen oxides (Nur *et al*., 2013).

Research on the utilization of corona discharge plasma in efforts to accelerate germination and growth has been conducted by Nurbuwati (2005), where radish seeds irradiated using corona discharge plasma were able to accelerate germination, increase hypocotyl length, and produce a sufficiently high germination percentage. Plasma is a rapid, economical, and pollution-free seed stimulation method that is relevant to developmental and physiological processes (Starek-Wójcicka *et al*., 2020). Plasma radiation on wheat seeds resulted in root length of sprouts twice as long as the control (Dobrin *et al*., 2015). Research on accelerating the germination and growth of TSS shallot seeds using plasma light has been conducted on shallot and garlic bulbs, but information on plasma radiation on TSS shallot seeds of Bima Brebes variety has not been conducted before.

2. MATERIALS AND METHODS

The research was conducted at the Plasma Laboratory of the Physics Study Program, Faculty of Science and Mathematics, Universitas Diponegoro, and Seed Viability Testing Laboratory at the Agricultural Technology Research and Assessment Institute (IP2TP Ungaran) located at Jl. BPTP No. 40, Paren, Sidomulyo, East Ungaran, Semarang Regency, Central Java, 50519. The research was conducted from March to April 2023. The design used was a Completely Randomized Design (CRD) with 6 treatments and 5 replications, totaling 30 research plots consisting of: P0 (without radiation), P1 (5 min of radiation), P2 (10 min of radiation), P3 (15 min of radiation), P4 (20 min of radiation), P5 (25 min of radiation). The True Shallot Seed used was of Bima Brebes variety.

2.1. Materials and Research Tools

The tools used in this research were Corona Discharge Plasma Reactor, Electrodes, Concentric Rings, Analog Multimeter, Digital Voltmeter, Power Supply, Refrigerator, Digital Scale, Thermometer, Hygrometer, Oven Camera, Writing Tools, Ruler. The materials used in this research were True Shallot Seed of Bima variety.

2.2. Plasma Light Treatment on Shallot TSS

In each treatment, 5g of TSS seeds were used. The weighed TSS seeds, 5g each, were then irradiated with 5 treatments and 1 control: P0 (without radiation), P1 (5 min of radiation), P2 (10 min of radiation), P3 (15 min of radiation), P4 (20 min of radiation), P5 (25 min of radiation). The plasma light was produced in the Plasma Laboratory in Building D of the Physics Study Program, Faculty of Science and Mathematics, Universitas Diponegoro. The plasma light was generated from a 11 V DC voltage source with a current of 50mA, i.e., the electromagnetic power or electric field arises due to the presence of direct current (DC) with very high voltage between positive and negative electrodes at a certain frequency, causing ionization of gas or air between two electrodes. The plasma light irradiation was performed with TSS shallot seeds placed on a sample plate with the seed position under the electromagnetic needle. The distance between the electrode unit and the seed sample plate was 3 cm. TSS shallot seeds that had been treated with plasma light were germinated in thinwall containers (2-liter plastic food containers). Germination was performed by sowing 10 TSS shallot seeds in 1 container/thinwall. The number of containers used in this research was 30 containers. The research results were then subjected to ANOVA (Analysis of Variance) test at a significance level of 95%, followed by DMRT (Duncan's Multiple Range Test) at 5%.

2.3. Data Analysis of Viability Parameters

Germination Power %. Observations were made on seeds that had germinated normally from the 1st observation (day 1) to the 7th observation (day 7) after sowing. Germination power was calculated using the ISTA (2010) formula:

$$\text{Germination Power (GP)} = \frac{Number\ of\ germinated\ seeds}{Number\ of\ seeds\ sown} \text{ x } 100\% \tag{1}$$

Seed Germination Rate. Seed germination rate (*GR*) indicates the ability of seeds to germinate quickly within a certain time range. *GR* is determined by counting the number of days required for the appearance of radicle and plumule. Seed germination rate was calculated using the Sutopo (2002) formula:

$$GR = \frac{N1T1 + N2T2 \ldots\ldots + NxTx}{Total\ number\ of\ seeds\ germinated} \tag{2}$$

where *N* is number of germinated seeds at a specific time, and *T* is time interval between the start and end of testing.

Seed Growth Rate (%/etmal). Seed growth rate (*SGR*) indicates the strength of seedling vigor, which is strong and able to withstand suboptimal environmental conditions. Seed growth rate is calculated daily for 7 days on normally growing seeds. Seed growth rate was calculated using the Tefa (2017) formula:

$$SGR = \sum_0^t d \tag{3}$$

where: t is germination time, and d is the percentage of normal germination.

Vigor Index. Seed vigor indicates the ability of seeds to grow rapidly, normally, and uniformly. Vigor index observations are made on the number of normally germinated seeds on the first count, i.e., on the 5th day according to ISTA (2010). Vigor index (*VI*) was calculated using the formula:

$$VI = \frac{N1}{H1} + \frac{N2}{H2} + \frac{N3}{H3} + \cdots + \frac{N_n}{H_n} \tag{4}$$

where *N* is the number of germinated seeds on the corresponding day, and *H* is corresponding day

Seedling Uniformity. Seedling uniformity is calculated based on the percentage of strong normal germination on the 6th day after sowing (DAS). Calculation for seedling uniformity of green beans, peanuts, and corn is done on the 6th day. Seedling uniformity (*SU*) was calculated using the Tefa (2017) formula:

$$SU = \frac{SS}{TS} \times 100\% \tag{5}$$

where *SS* is the number of strong seedlings, and *TS* is the total seeds analyzed.

Seedling Length. Seedling length is measured when the plant is 12 days after sowing (DAS) and measured using a caliper.

3. RESULTS AND DISCUSSION

The results of ANOVA (Analysis of Variance) test at a significance level of 95% indicate an influence on Germination Power, Seed Germination Rate, Seed Growth Rate, Seedling Uniformity, and Seed Germination Rate of shallots originating from True Shallot Seed (TSS). The True Shallot Seed (TSS) of red shallots irradiated with plasma for 15, 20, and 25 min showed an average germination power of up to 98%, while P0, P1, and P2 exhibited average germination powers of 48.00% and 76.00%. The response of seed germination power to plasma light radiation varied among TSS of red shallots. Generally, plasma radiation yielded the best results in improving germination power in the range of 15 to 25 min for seeds originating from bulbs (Puspitasari, 2018). According to Măgureanu *et al*. (2018), seeds treated with plasma showed faster germination power compared to those untreated.

Table 1. Results of average analysis of germination power, seed germination rate, seed growth rate

Variable	Unit	Treatment					
		P0	P1	P2	P3	P4	P5
Germination power	%	48.00^{d}	76.00^{c}	72.00^{c}	98.00^{a}	92.00^{b}	94.00^{b}
Seed germination rate	%	1.08^{d}	1.80^{c}	1.84^{bc}	1.96^{a}	1.94^{a}	1.88^{b}
Seed growth rate	%/etmal	2.00^{c}	3.20^{b}	3.23^{b}	5.00^{a}	3.33^{b}	4.90^{a}

Note: Different superscript letters in the same row indicate significant differences between treatments in the DMRT test with 95% significance level.

From the observations, plasma radiation for 15, 20, and 25 min demonstrated the best average seed germination rates of 1.96%, 1.94%, and 1.88%, respectively. The control exhibited an average seed germination rate of 1.08%, while seeds irradiated with plasma for 5 and 10 min showed germination rates of 1.80% and 1.84%, respectively. This is consistent with research (Nur *et al*., 2013; Măgureanu *et al*., 2018) indicating that the germination rate of TSS of red shallots irradiated with plasma for 15 min increased and significantly affected germination.

Radiation treatments of 15, 20, and 25 min also influenced the seed growth rate. Each treatment yielded average %/etmal rates of 5.00 for P3, 3.33 for P4, and 4.90 for P5 treatment. Based on data from Table 1, there was a non-significant decrease in the 20-min (P2) and 25-min (P3) radiation treatments. This is because longer plasma light radiation affects the quality of seed and damages the cell tissue due to the enzymes and proteins present in the seeds. According to Ariyanti *et al*. (2020), the longer duration of plasma radiation affects the enzymes in the seeds, as enzymes are composed of proteins that are sensitive to high temperatures. Cold plasma can be produced on a laboratory scale using the Dielectric Barrier Discharge (DBD) technique, which can generate various atom or molecule species when interacting with air, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) including NOx, OH, O, and O_3. Reactive oxygen species (ROS) are involved in plant development processes by acting as signaling molecules for cell proliferation and differentiation, seed germination, and root hair growth. Reactive nitrogen species (RNS) are known as signaling molecules that control plant development processes, activating phytosensors and phytoactuators in plants and seeds. Phytoactuators are parts of plant tissues responsible for controlling responses originating from electrochemical energy sources or hydraulic pressure, while phytosensors are defined as parts of plant tissues that can detect, record, and transmit information related to physiological processes in plants (Volkov *et al*., 2019).

Nitrogen infiltrates the seeds and directly binds in the form of N^{2+} or N^{+} ions from the dissociation process followed by ionization or molecular ionization processes (Nadzifah & Prihastanti, 2019). With the diffusion of nitrogen ions into the seeds, the nitrogen content in the seeds increases, supporting germination and growth. According to Agurahe *et al*. (2019), the entry of water into the seeds via imbibition is responded to by the embryo by releasing GA (Gibberellin), as a response to the entry of water into the seeds. Gibberellin released spreads throughout the seed and enters the aleurone particles beneath the seed coat. The aleurone particles then respond by forming the alpha-amylase enzyme through protein synthesis in response to gibberellin. Alpha-amylase works to hydrolyze the endosperm, breaking down the endosperm into monomers, and the starch present in the endosperm is used by the embryo for germination. Consistent with the research by Puspitasari (2018), the optimal time for plasma irradiation is 15 min.

Table 2. Results of average analysis of vigor index, seedling uniformity, and seedling length. (%)

Variable	Unit	Treatment					
		P0	P1	P2	P3	P4	P5
Vigor index	-	2.00^{d}	3.20^{c}	3.23^{c}	4.90^{a}	3.80^{b}	4.80^{a}
Seedling uniformity	%	42.00^{d}	74.00^{c}	72.00^{cd}	100.00^{a}	90.00^{b}	92.00^{b}
Seedling length	cm	1.09^{e}	1.20^{d}	1.38^{c}	1.76^{b}	1.75^{b}	1.90^{a}

Note: Different superscript letters in the same row indicate significant differences between treatments in the DMRT test with 95% significance level.

In Table 2, the vigor index variable shows that out of the 6 treatments conducted, the highest average vigor index value of 4.90 (P3) was obtained. The lowest average vigor index value was 2.00 (P0). The control treatment had a lower value compared to P3, P4, and P5, indicating that longer durations of plasma light radiation provide a good increase in the vigor index value for TSS of Bima Brebes red shallot bulbs. The vigor index represents the percentage of normal germination on the first count, indicating the percentage of seeds that germinate quickly (Junaidi *et al*., 2018). The results of the vigor index test are closely related to seed quality. Seeds that germinate quickly will be better able to cope with suboptimal field conditions (Widajati *et al*., 2013).

Seedling uniformity was observed based on the number of normal germinations counted in the middle of the germination observation period, where strong normal germinations exhibit a better and more complete germination structure than the average of other normal germinations. Based on the results of the DMRT test with a significance level of 95%, the control treatments (P0), (P1), and (P2) showed significant differences compared to parameter (P3) with a 15-min irradiation time (Table 2).

High seedling uniformity indicates strong growth vigor because a group of seeds showing synchronous and strong growth will have high growth vigor. Plasma release produces reactive neutral species, charged species (electrons, ions), electric fields, and ultraviolet radiation. These factors cause changes in the density of reactive oxygen species (ROS), reactive nitrogen species (RNS), pH, oxidation-reduction potential, electrical conductivity, and so on, and affect seed germination, plant growth, and crop quality (Ohta *et al*., 2016). N_2 molecules produced from plasma reactors become reactive (such as ammonia or nitrate) through nitrogen fixation processes in the form of NOx (Bormashenko *et al*., 2015). The nitrogen fixation process from the air by breaking down N_2 and O_2 molecules into N and O atoms that form nitrogen oxide (nitric acid or ammonia).

From the data analysis results (Table 2), treatments (P0) 1.09, (P1) 1.20, and (P2) 1.38, showed significantly different values compared to treatments (P3) 1.76, (P4) 1.75, and (P5) 1.90. Seeds have four layers: cuticle, epidermis, hypodermis, and parenchyma. The role of seed coat as a water modulator; it controls the entry of water so that it can be absorbed slowly by the cotyledon to minimize or avoid damage during imbibition. Pawlat *et al*. (2018) state that plasma treatment can alter seed structure by removing the upper cuticle layer coated with wax. The exothermic reaction of plasma releases heat around 37 °C and can melt or erode wax by reactive oxygen species (ROS) and reactive nitrogen species (RNS) and introduce N ions into the embryo of red shallot TSS seeds. The erosion of the wax layer creates an etch effect, from which etching effect will create roughness on the seed coat surface, providing space for water molecules to be absorbed by increasing hydrophilicity. If the cuticle layer is degraded, water absorption can penetrate further into the embryo seed. Consistent with research by Tridyaksa (2007); Bormashenko *et al*. (2015), that the release of corona glow plasma radiation generated from two electrodes containing nitrate ions (NO_3^-) can increase hydrophilicity and water permeability.

Figure 1 shows the results of plasma radiation testing on the germination of onion seeds. Based on the figure, there is a noticeable difference in radicle length across the different treatments. From the first to the third day, treatments P3, P4, and P5 exhibited longer radicles compared to treatments P0, P1, and P2. Over the 72-hour period (3 days) shown in Figure 1, it is evident that 15 minutes of radiation resulted in better seedling growth compared to the control (0 minutes), 5 minutes, and 10 minutes of radiation. However, the results for 20 minutes and 25 minutes of radiation were similar to those for 15 minutes. The factor influencing the differing outcomes among the plasma radiation treatments is the availability of nitrogen. Nitrogen is essential for all organisms, particularly for the vegetative growth of plants such as onions. According to Nadzifah & Prihastanti (2019), the four most abundant elements found in plant tissues are C, H, O, and N.

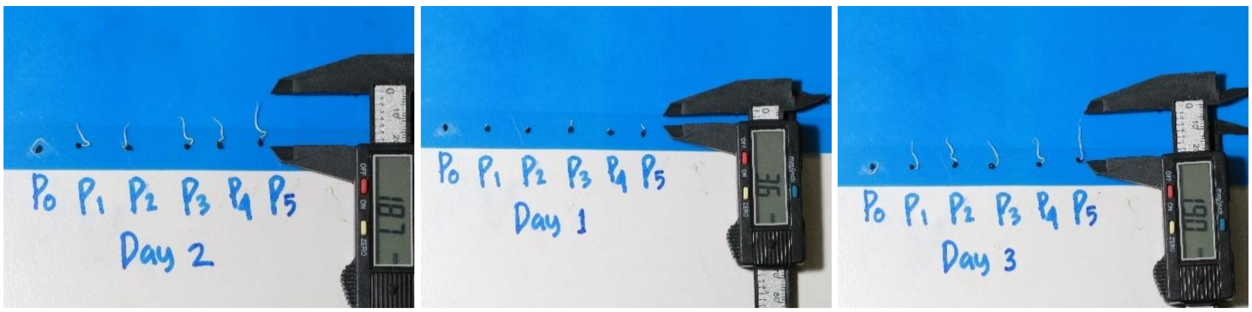

Figure 1. Development of seedling length: first day (left), second day (center), third day (right)

4. CONCLUSION AND RECOMMENDATION

The application of plasma light for 15 min increases seed germination power, vigor index, seedling growth rate, seedling uniformity, seed germination rate, and seedling length of TSS of red shallots. The use of TSS still faces challenges in cultivation, so it needs to be continued with planting in the field with the same treatment to determine the growth and harvest results of red shallots. Thus, from the growth and harvest results, it is expected to serve as a reference for the cost of red shallot farming, especially for red shallot farmers.

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