The effect of low-pressure plasma treatment on sunflower seed germination and sprouts growth rate

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Abstract. This study presents the influence of low-pressure plasma treatment on germination behaviour of sunflower seeds (\textit{Helianthus annuus}). Both peeled and unpeeled seeds were treated with Ar plasma. The plasma was characterized using OES for understanding of physical and chemical species in the plasma. The physical properties and chemical properties of the samples were studied using SEM and water contact angle. Growth parameters including water uptake, seed germination, sprouts length and dry weight were also investigated. It was found that the surface roughness increased with the plasma-generating power. The water absorption decreased when the power increased and super hydrophilicity was achieved at a 100 W. The water uptake rate showed highest at 150 W plasma power. The highest rate of seed germination was found when the sunflower seeds were treated with plasma power of 150 W at 5-day sowing. Sprout length and dry weight increased with the plasma power as well. In summary, sunflower seeds treated by Ar plasma could improve seed germination rate, leading to significant change in wettability and growth parameter.

1. Introduction
Sunflower (\textit{Helianthus annuus}) is a flower that is particularly suitable to be planted in a drought-like climate in Thailand. Because sunflower is easy to grow, grows fast, likes hot and dry air and furthermore to planting a wide area to be beautiful field as a tourist attraction. Sunflower is also an important economic crop of Thailand as well. Moreover, the critical air pollution of particulate matter less than 2.5 micrometers (PM 2.5) in the north of Thailand is higher than the standard and is harmful to health. Thai PBS website reports that the cause of PM 2.5 comes from open burning 54%, up to 209,937 tons per year, manufacturing industry 17%, transportation 13%, electricity production 9%, and activities in residential or commercial businesses 7%. Mekong region included the upper north of Thailand is a single plant cultivation area, especially corn that is the one cause of open burning, to serve the food production supply chain of a large agro-industrial company. Sunflower is the other plants to replace the corn growing area to sell seeds as crops instead of growing corn like a farmers in Saraburi and Lopburi. However, the problem of seed infection and seed germination rate are causes farmers to waste the seeds for planting in the large areas. Therefore, to reduce the cost of planting, the seeds should be sterile and has a high germination and survival rate. Non-thermal plasma has been used to decontaminate seeds from pathogens, to improve their germination properties and length of sprouts [1-4]. The main factors
of plasma improvement of germination are increase in the water uptake through a partially etched seed coat due to the plasma treated seeds become a hydrophilic surface [2]. Normally, the germination rate and growth yield of seeds are enhanced by chemical methods that lead to genetic dissimilarity and cause negative effects to life and nature. In this study focus on the organic trend, plasma technique is the right choice.

2. Materials and methods

2.1. Materials

Sunflower seeds cultivar “Striped seeds” were chosen for investigation to the effect of plasma technique. The sunflower seeds were setup into two groups, peeled and unpeeled seeds, each conditions have 25 seeds.

2.2. Plasma treatment

The argon plasma treated were carried out by using a self-made 13.56 MHz inductively coupled plasma reactor. The detail of the reactor was described elsewhere [5]. The samples were put into a quartz cylinder then the cylinder was evacuated to a based pressure of 0.01 Pa. The peeled and unpeeled seeds were treated with RF powers of 75 W, 100 W, 125 W and 150 W. The treatment time was fixed at 2 minutes with an argon gas pressure at 1.65 Pa. Optical emission spectroscopy (OES) was used to observe the active plasma species species and the changes in their relative peak intensities, as the RF powers were varied. The plasma radiation collected by the lens was focused onto the optical fiber to an input of S2000 Miniature Fiber Optic Spectrometer, Ocean Optic, Inc. and measured from 200 to 800 nm in 0.6 nm steps.

2.3. Surface Characteristics

2.3.1 Surface morphology. Surface morphology of the samples was observed by scanning electron microscopy (SEM) (JEOL JSM-5910 LV; 15 keV)

2.3.2 Contact angle. The static contact angle was observed by using 2 µl of de-ionization water at room temperature. Fifth replicates of each condition of treatments were analyzed.

2.4. Growth parameters studied

2.4.1. Seed imbibition. Water uptake was measured at 2, 4, 6, 8, 10, 12 and 24 hrs. from the beginning of the experiment. Seeds were weighted prior to the imbibition as well as at the indicated time points and water uptake was calculated based on the weight differences.

2.4.2. Seed Germination. Germination test is used to determine seed viability. The germination test was conducted at room temperature. 25 seeds were taken from each conditions then placed in the Petridis. The daily record of germinated seed was taken up to 5th day.

2.4.3. Growth of seedlings. Seedlings were measured in height and dry weight. After day 5, sprouts were seeded and measured the length from stem to shoot, daily. In day 12 sprouts were dried in an electric oven at 80 °C for 24 hrs. and measured for dry weight.

3. Results and discussion

3.1. Plasma Diagnostics

The spectrum of Ar plasma was observed by OES in a range of 200-800 nm. The prominent lines observed were the OH line at 308 nm, the N₂ line at 420 and 450 nm, the O₂ line at 556 nm, the H₂ lines at 603 nm and the Ar lines at 651 to 843 nm. The intensity was increased with increasing RF power, as demonstrated in figure 1. These reactive oxygen and nitrogen species (RONS) played an important role in sterilization.
3.2. Surface Characteristics

3.2.1. Surface morphology. The surface morphology of peel and unpeel seed coat before and after plasma treated have a slightly change (as shown in table 1). The rising powers of the plasma cannot see the significantly changing in the surface roughness.

Table 1. The surface morphology of peeled and unpeeled seeds with control 75 W, 100 W, 125 W and 150 W.

|                   | Control          | Plasma treated |
|-------------------|------------------|----------------|
|                   |                  | 75W | 100W | 125W | 150W |
| Unpeeled seeds in |                  |     |      |      |      |
| 100X              |                  |     |      |      |      |
| Unpeeled seeds in |                  |     |      |      |      |
| 5000X             |                  |     |      |      |      |
| Peeled seeds in   |                  |     |      |      |      |
| 100X              |                  |     |      |      |      |
| Peeled seeds in   |                  |     |      |      |      |
| 5000X             |                  |     |      |      |      |

3.2.2. Contact angle. The contact angles were obtained by the static sessile drop techniques. After plasma treated, water contact angles both of peeled and unpeeled seeds were decreased from 108.45 to 0 degree as shown in figure 2. The water absorption decreased when the power increased and super
hydrophilicity was achieved at a 100 W. The higher power made a lite roughness on the surface, that is the cause of hydrophilic properties. The contact angle is the quality to its hydrophilic ability, which is relative to the germination seeds. An increasing of the polar components in surface energy increased greatly as the result of the incorporation of polar groups on the surface.

Figure 2. Water contact angle of the sunflower seed which treat/untreated plasma. (a) is unpeeled seed and (b) is peeled seeds.

3.3. Growth parameters studied

3.3.1. Seed imbibition. The plasma treated sunflower seed effects in the different powers. The water uptake rate of the unpeeled seeds has a low rate nevertheless peels seeds have a higher. The water uptake rate of peeled seeds showed highest at 150 W plasma power. The result shown in figure 3.

Figure 3. Effect of plasma on the water uptake of sunflower seeds from 0 to 24 hrs. of imbibition. (a) is unpeeled seed and (b) is peeled seeds.

3.3.2. Seed Germination. The result of the seeds germination percentage was shown in figure 4. Unpeel seeds treated under plasma power 75 W was slightly high in a seed germination percentage but then increased the plasma generator-power, it seemed like that the seed germination percentage was decreased. For the peel seeds treated the low plasma generator-power non effect on the germination but it is significant stimulatory at plasma power 150 W.
Figure 4. Effect of plasma on the seed germination percentage. (a) is unpeeled seed and (b) is peeled seeds.

Figure 5. Effect of plasma on the growth in length of shoot after plant 7 days (day 6th to 12th). (a) is unpeeled seed and (b) is peeled seeds.

Figure 6. The seeding weight and dry weight of sunflower seed with 80°C in the oven for 24 hrs. (a) is unpeeled seed and (b) is peeled seeds.
3.3.3. Growth of seedlings. All the plasma treatments had a significant stimulatory effect on the germination and vigor. The growth of seeding better and faster and produced longer and heavier seedling. The seed treated by plasma power 150 W has highest value in length of shoot seedling both of peeled seeds and unpeeled seeds. The result shown in figure 5. The growth in length of shoot seedling, that treated by plasma has higher than the control. Figure 6 shown the seeding weight and dry weight of sunflower seeds, there are in the same trend as the length of shoot seeding.

4. Conclusions
The low pressure plasma treated on both of peeled seeds and unpeeled seeds effective to seed germination rate, leading to significant change in wettability and growth parameter. The RONS provoked the oxidation lipids present in seed coats leading to hydrophilic property which promoted seed water uptake and seed germination.

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