Stimulation of Seed Germination and Physiological Development in Plants by High Voltage Plasma and Fine Bubbles

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Abstract—The effects of high voltage plasma (HVP) and air fine (micro/nano) bubbles (FBs) on seed germination and seedling growth were investigated for Chinese celery (*Apium graveolens* L.) and sweet corn (*Zea mays* L.), respectively, followed by the second experiment to study further the effect of FBs on growth and fruit quality in melon (*Cucumis melo* L.). The results indicated that all the treatments of HVP+FBs, HVP and FBs greatly improved Chinese celery and sweet corn seed germination rates when compared with conventional distilled water. Moreover, the HVP and FBs treatments were found to achieve the longest root growth. For sweet corn seedlings, treatments of HVP+FBs, HVP and FBs have also increased root length. Furthermore, the melon experiments showed that FBs could improve leaf width, length, and number, and also increase the number of flowers per vines compared with untreated plants. However, fruit quality was found similar, while the leaf, stem and total dry matter have increased in weight in the FBs treatments.

Keywords—High voltage, plasma, fine bubbles, seed germination, treatment

I. Introduction

Plasma is an ionized gas, the fourth state of the matter. High voltage plasma (HVP) is now being widely used in many fields such as science, industry, agriculture, medicine, waste water treatment and sterilization [1,2]. The plasma irradiation for a few tens of minutes per day was reported to increase the growth rates of Chinese cabbage by supplying HVP-produced nutrients, and to inactivate bacteria [3].

As for fine bubbles (FBs), they consist of micro bubble (MB) of 1-100 μm in diameter, and ultra-fine bubble (UFB) as less than 1μm [4]. FBs are gas bodies surrounded by water, being used in many fields of biological and scientific fields, and also in the application fields, such as waste water, medical and food industry [5].

In many crops, HVP and FBs technologies have been reported to enhance seed germination and plant physiological growth, where many researches showed successful results in laboratories and field experiments [1,2]. In Thailand, recently both technologies have been introduced to produce extraordinary effects in agriculture and aquaculture, since Thailand belongs to the tropical and subtropical climates, and can make Thailand more appropriate for the rapid growth and development of many crops, as well as sterilize many kinds of microorganism such as pathogens. Actually, Thai crop farming has many problems, such as insects, pathogens, flooding, and water deficit. In this respect, both technologies are expected to be very useful for crop production, particularly, improvement of seed germination, acceleration of the growth rate of the crop, and control of pests and pathogens.

The experiments made so far have shown that HVP can promote seed germination, i.e., the cold plasma treatment can improve the germination, promote the growth and increase the physiological level of wheat [6]. Moreover, HVP plasma technology is found to promote plant growth through the study that bubble discharge in water increased growth rate of Japanese mustard spinach [3].

It is found that FBs could induce vegetative growth, increase leaf and stem dry matters in the water with FBs, and highly dissolved oxygen (DO) are important for initiation of seed germination. It has been shown that FBs can promote seed physiological activities and activate germination-related enzyme in barley seed [7]. Moreover, FBs are found to enhance plant growth and barley's germination rate and increase dry weight in lettuce [7,8]. Also, it was found that the microbubble generation in a nutrient solution can promote rapid lettuce growth [9].

In the previous studies, more detailed findings were reported on FBs. It was found that FBs can add more air and oxygen to the water which is required for respiration to oxidize starches, fats, and other food reserves to induce metabolic activity in seeds [10]. Also for Barley seeds treated with gas-mixed nano bubble water and 0.3 mM H_2O_2, germination rates at each observation time of the seeds were found greater than those submerged in distilled water [11]. In gas FBs compounded with superoxide anion radicals (O_2), hydrogen peroxide (H_2O_2), and hydroxyl radical (・OH), it was found that oxygen and H_2O_2 had the effect of inducing seed germination [12,13].

Since Chinese celery, sweet corns and melons are economic crops in Thailand, seed germination as first step of crop growth and development is a very important issue, in particular, if HVP and FBs could reduce the time required for seed germination with proficient survival rate for seedlings and further crop establishment.

Furthermore, in case FBs could also promote favorable growth of melons, which needs longer farming time, this FBs method would be preferably adopted due to rapid economic returns.

The purpose of this study is to evaluate and confirm the effects of HVP and FBs on seed germination in Chinese celeries and sweet corns, and also how FBs influence the vegetative and reproductive growth of melons.

II. Methodology

In the first short-term experiment, the seeds were treated with HVP device (through 75 holes of 0.75mm diameter of a quartz pipe of 30 mm diameter, 200 mm lenght with 5L/min

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air flow, 15 kV pulse discharge was applied with 15.64 KHz) and air FBs (KVM-01; water flow rate 1.7 L/min, air flow rate 0.1 L/min, operation pressure 0.25-0.4 MPa and 45W pump, having air nano bubbles in the range of 10E9 bubbles/mL with a peak around 200 nm measured by Horiba-960A laser bubble analyzer) developed by RMUTL, Chiang Mai, Thailand (Fig.1) [14,15].

The first germination experiment was made for 1,600 Chinese celery seeds (Benjamitr Enterprise Co. Ltd., Nonburi, Thaiand), and 800 sweet corn seeds (Sunsweet Public Co.Ltd, Chiang Mai., Thailand). The treatments consisted of 1) distilled water, 2) 10L FBs water operated for 10 minutes, 3) 10L HVP water operated for 10 minutes, and 4) 10L FBs water for additional 10 minutes operation, for the 10L HVP water already treated for 10 min, respectively.

The Chinese celery seeds were soaked for 12 hours for each treatment, and then placed on germination test papers, followed by 4 kinds of 50 mL “treated water” supply every day.

In the sweet corn experiment, 800 sweet corn seeds were soaked for 12 hours for each trement, and then put in a media (Peat moss) and supplied 4 kinds of 100 mL “treated water” every day.

The experiment was made in the laboratory room, and after put on germination papeand media, they were moved to the greenhouse since second days. The data on seed germination percentage and the period of germination were recorded, and plant height and root length were also measured after 24 days of treatment for Chinese cerelies, and 8 days for sweet corns. Plant heights were measured from the connecting point between the stem and the root to the terminal shoot, and root lengths were measured from the connecting point between the stem and the root to the terminal root, respectively. The average air temperature and relative humidity during experiments were controlled in the range of (29.8±1.5) ºC and (57.8±5.3) %, respectively.

In the second experiment of melons, considering the 75 long days required for harvesting, and practical applications of the new technology by Thai farmers, we focused this time only on easier air FBs.

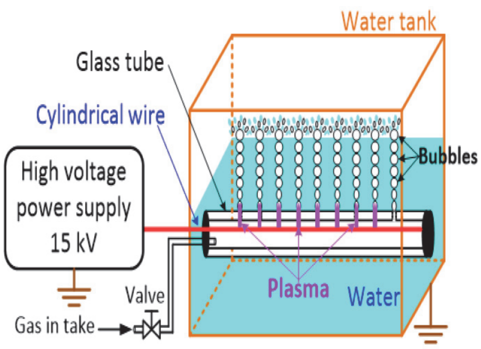
‘Pot Orange’ melon plants (Known You Seed, Chiang Mai, Thailand) were treated twice a week with each 10 L air FBs water which was treated for 10 minutes.

Four kinds of treatments were applied, i.e. 1) 0.5× nutrient solution concentration (NSC) with FBs, 2) 0.5× NSC without FBs, 3) 1× NSC with FBs, and 4) 1x NSC without FBs. Two kinds of nutrient solution were prepared; half of normal concentration (0.5×), and normal concentration (1×), supplied from Hoagland and Arnon formular[16], and nutrient solution pH was adjusted to be 6.5 by addition of H_2SO_4. The melon plants have then grown in black plastic pots with substrate culture of chopped coconut bark and coconut husk of equal ratio, and nutrient solution of 1.5 L/day was supplied by drip irrigation everyday from a 40 L container.

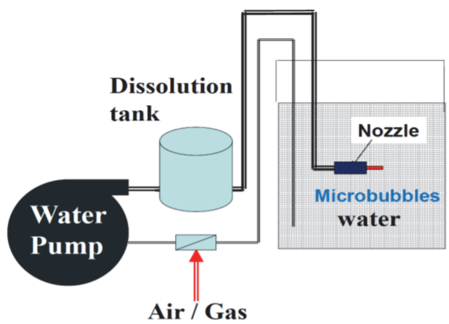
In melon experiments, the following parameters were measured and recorded, i.e., plant height, stem diameter, leaf number, leaf width, leaf length, leaf chlorophyll content. At the end of the experiment, the dried weight of the plants was also measured as a dried matter.

All parameters were subjected to statistical analysis of variance (ANOVA). Statistical differences with *p*-values less than 0.05 were considered significant, and the means were compared by well-known Duncan’s new multiple range test (DMRT) [17].

Both experiments were conducted in a greenhouse unit at Agricultural Technology Research Institute, Rajamagala University of Technology Lanna, Lampang, Thailand.



(a)



(b)

Fig.1. HVP (a) and FBs generator (b) developed at RMUTL.

III. Results

The first experiment showed that averaged seed germination percentage of Chinese celery seeds in thecase of 4) HVP+FBs treated water was found to be greater than 1) distilled water (Fig.2). However, the period to germination saturation was hastened by 2-3 days for HVP with FBs when compared with others (Fig. 2).

After 24 days it was found that 4) HVP+FBs treatment produced the longest root growth, while the average plant height was comparable (Fig.3). For sweet corns, they showed,

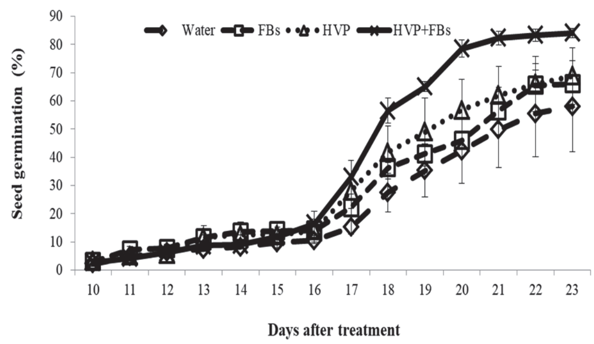


Fig. 2. Enhanced germination of Chinese celery seed germination watered with HVP and FBs.

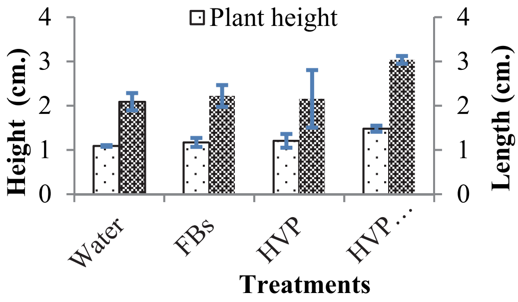


Fig. 3. Plant height and root length of Chinese celery seedling at 24 days after treatment

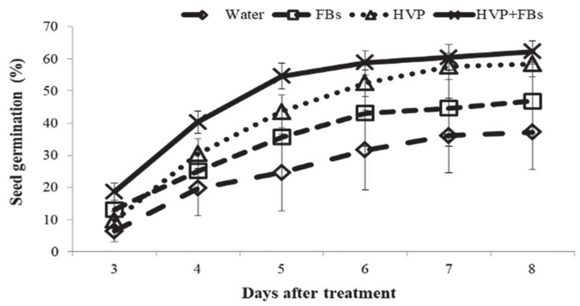


Fig. 4. Effect of water, FBs, HVP and HVP+FBs on seed germination of sweet corns

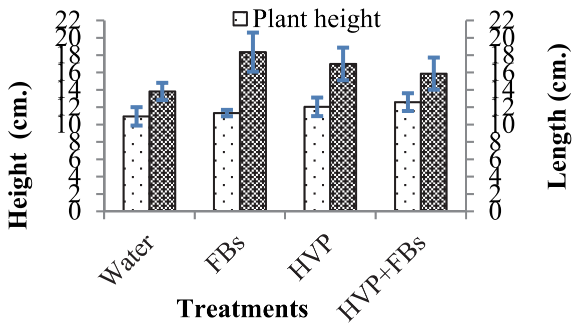


Fig. 5. Plant height and root length of sweet corn seedling at 8 days after treatment with water, FBs, HVP and HVP+FBs.

4)HVP+FBs, 2)HVP and 3)FBs provided better seed germination percentage than 1) distilled water (Fig.4). However, it was found that HVP+FBs had no effect on average plant height in sweet corn seedlings, while root length by FBs, HVP, HVP+FBs were found greater (Fig.5).

The second melon experiment was initiated to see what effects of air FBs water can have on physiological changes in melons. Results revealed that the water of 1×NSC with air FBs produced greater leaf width, leaf length, leaf number and number of flowers per vine than others (Table I). Eventually, FBs were found not to have any marked effects on fruit weight, fruit width, fruit length, fresh thickness, or total soluble solid (TSS) as seen in Table II.

Also, the water of 1×NSC with FBs was found to produce heavier dried leaves, stems and total dry matters than others, while the water of 0.5×NSC had the lowest effect (Table III). Dissolved oxygen (DO), temperature (T), electrical conductivity (EC) and pH of nutrient solution in the

TABLE I
EFFECT OF FBS ON LEAF GROWTH, LEAF GREEN COLOR AND FLOWER NUMBER OF MELON PLANT

Treatments	Leaf width (cm.)	Leaf length (cm.)	Leaf number/ vine (leaves)	Flower number/ vine (flower)
0.5× NSC with FBs	**13.40 b***	**12.50 bc**	**30.00 b**	**16.48 b**
0.5× NSC	**13.40 b**	**11.90 c**	**29.20 bc**	**13.95 c**
1× NSC with FBs	**14.80 a**	**13.70 a**	**32.80 a**	**18.88 a**
1× NSC	**13.90 b**	**13.00 b**	**31.60 ab**	**17.63 ab**
F-test	*	*	*	*

* Mean within column with different alphabets differ significantly at P<0.05 by DMRT,
ns = Non-significant

TABLE II
EFFECT OF FBS TREATMENT ON FRUIT CHARACTERISTICS OF MELON AFTER HARVEST

Treatmen ts	Fruit weigh t (g.)	Fruit width (cm.)	Fruit length (cm.)	Fresh thickness (cm.)	TSS (°Brix)
0.5× NSC with FBs	**879.8**	**11.46**	**11.82**	**2.36**	**16.8**
0.5× NSC	**897.2**	**11.66**	**11.94**	**2.52**	**16.6**
1× NSC with FBs	**880.6**	**11.62**	**11.84**	**2.43**	**17.2**
1× NSC	**882.2**	**11.6**	**11.84**	**2.37**	**16.8**
F-test	**ns**	**ns**	**ns**	**ns**	**ns**

ns = Non-significant

experiment were found that DOs in both 0.5× or 1×NSC with FBs were higher than untreated case. Temperatures in nutrient solution were kept in the range of 28.3-29.0 °C, EC of 0.5×NSC was found to be 1.51-1.56 and 1×NSC 3.02-3.04, respectively, and pH of 0.5×NSC 6.52-6.56, and 1×NSC 6.51-6.58, respectively (Table IV).

IV. DISCUSSION

In the experiments of Chinese celery and sweet cones, HVP and FBs were found to have positive effects on the seed germination rates. In addition, HVP+FBs promoted root growth in Chinese celery seedlings while they had no effect on plant height. For sweet corn, FBs were found to enhance root growth of seedlings. It was also found that air FBs could stimulate leaf growth and flower production in melons.

In this study, we could find many specific advantageous phenomena, and confirm the preliminary results obtained previously, in which HVP, FBs and HVP+FBs can enhance seed germination in Chinese celery and sweet corn as well as HVP can inactivate bacterial growth in nutrient solutions [18]. Furthermore, HVP induced seed germination was considered

TABLE III
THE DRY MATTER OF LEAF, STEM, ROOT AND TOTAL DRY MATTER IN MELON AT THE END OF EXPERIMENT

Treatments	Dry matter (g.)			
	Leaf	Stem	Root	Total
0.5× NSC with FBs	26.42 bc*	11.20 bc	1.52	39.14 bc
0.5× NSC	24.96 c	10.58 c	1.46	36.39 c
1× NSC with FBs	31.64 a	12.79 a	1.63	45.27 a
100% NSC	30.00 ab	12.00 ab	1.57	42.38 ab
F-test	*	*	ns	*

* Mean within column with different alphabets differ significantly at P<0.05 by DMRT, ns = Non-significant

TABLE IV
DISSOLVED OF OXYGEN (DO), TEMPERATURE (T), ELECTRICAL CONDUCTIVITY (EC) AND PH OF NUTRIENT SOLUTION

Treatments	DO ($mg \cdot L^{-1}$)	T (°C)	EC (µS/cm)	pH
0.5× NSC with FBs	6.50 a*	28.95	1.51	6.52
0.5× NSC	5.67 b	28.28	1.56	6.56
1× NSC with FBs	6.48 a	28.91	3.02	6.51
100% NSC	5.52 b	28.33	3.04	6.58
F-test	*	ns	-	ns

** Mean within column with different alphabets differ significantly at P<0.05 by DMRT,
ns = Non-significant

to be probably and partially due to surface casing ablation and transmission of oxygen and water through the seed coat to the embryo [1,19], and atmospheric-pressure plasma to be able to induce seed germination, seed decontamination and inactivate microorganism as well.

In addition, reactive oxygen species (ROS) and other oxidizers in combination with lowering pH could have decreased pathogens in water and soil in our experiments [20], since HVP can produce also atomic oxygen (O), ozone (O_3), hydroxyl radical (• OH), atomic nitrogen (N), nitrogen oxide(NO_x), nitrite(NO_2), nitrate(NO_3) and hydrogen peroxide (H_2O_2) [21,22]. Although there are many factors reported, i.e., control of seed germination consists of moisture, temperature, oxygen and light [10], and NO_3 and H_2O_2 can induce also seed germination, since NO_3 can act as a source of N and a seed germination enhancer [23].

Our results were found, basically, to follow these results of Sivachandiran and Khacef (2016) who suggested that atmospheric pressure cold air plasma has positive effects on seed germination and seedling growth of such as radish, tomato and sweet pepper plants [24].

On the other hand in our experiments, air FBs were found to enhance seed germination and root growth in Chinese celery and sweet corn, as well as leaf growth and dry matter in melon, which are in good agreement with previous studies.

In melon seedling experiment, it was found that treated air FBs increased the dissolved oxygen concentration in the nutrients solution, and subsequently it has promoted leaf growth and dry matter production. However, there was no increase in fruit growth or quality, although there are many reports about the positive effects of FBs stimulating plant growth, showing nano and micro bubbles can promote the growth of leaf lettuce compared with air-macrobubbles [25]. In our melon experiment, air FBs supply twice a week may be probably not sufficient enough to outweigh the effect of melon development under the temperature of nutrient solution above 28ºC in the green house.

V. CONCLUSION

In this study, it was found that HVP, FBs and HVP+FBs could induce germination of Chinese celery and sweet corn seeds. In particular, HVP+FBs could enhance root length in Chinese celery greatly, while there was no effect on the plant's height. However, both HVP and FBs were found to increase root length in sweet corn seedlings.

Moreover FBs in nutrient solution of melon seedlings were found to improve leaf width, length, and number, and also increase the number of flowers per plant compared with untreated vines, although FBs did not affect the fruit quality in melon. Furthermore, the leaf, stem and total dry matter were found to increase in weight for plants treated with FBs. Finally, it is expected that it would bring greater benefit to Thai farmers to apply HVP, FBs and HVP+FBs in enhancing seed germination for their crop production.

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