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Full Length Article

Research on Generation of Negative Air Ions by Plants and Stomatal Characteristics under Pulsed Electrical Field Stimulation

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Abstract

As an important indicator of air quality negative air ions (NAI) is good for human psychological and physiological condition. Under natural conditions, plants generate low amounts of NAI and frequently it cannot meet the requirement for improving physical health. However, the capacity of NAI generation by plants was significantly enhanced with stimulation of pulsed electric fields (PEF). To study the effects of PEF and light stimulation on NAI generation by plants, we choose the *Crinum asiaticum* var. *sinicum* (poison bulb), *Narcissus tazetta* var. *chinensis* (narcissus) and *Zephyranthes carinata* (zephyranthes) which generate more NAI than other plants under natural conditions. The results showed that different parameters of PEF have different influences on NAI generation by plants. Thus each plant had the optimal parameters of PEF to generate NAI efficiently. When the optimal parameters of PEF were applied the higher voltage the plant had the more NAI it generated. Furthermore and the capacity to generate NAI dramatically improved with the strengthened light intensity. In the absence of electrostimulation the NAI concentration increased slightly as the light intensity strengthened, but there was no considerable effect of the light intensity on the plant's capacity to generate NAI. Additionally, there was a close correlation between the capacity of the plant to generate NAI and the characteristics of leaf stomas. Under the stimulation of the optimal parameters of PEF, with its wider opening, the stomata had a greater density, thus the plant generated more NAI. The capacity of plants to release NAI was influenced by such elements as PEF, light intensity and characteristics of plants. Among these factors the PEF had a more considerable effect than other factors on the plant. Hence, to make plants generate NAI efficiently, the best way is to use the optimal parameters of PEF and at the same time made leaf stomata open to a certain degree.

Keywords: Plants; Negative air ions; Electrostimulation; Pulsed electric field; Light intensity; Stomata

Introduction

The biological and ecological effect of negative air ion (NAI) have been widely recognized. As an important indicator of air quality as well as the contribution to physical health (Krueger and Reed, 1976; Krueger, 1985; Takahashi *et al*., 2008; Kolarz *et al*., 2009), the NAI is well known for 'air vitamin' (Li, 1996; 1999). There are three ways to generate NAI in nature environment. Firstly, atmospheric molecules, affected by such factors as cosmic rays, thunder and lighting, ionize to generate NAI. Secondly, the Lenard effect makes water molecules decompose to generate NAI. Thirdly, the photoelectric effect caused by the discharge of forest canopy and leaf apex as well as photosynthesis of green plants ionizes the air thus generating NAI (Tikhonov *et al*., 2004; Liang *et al*., 2014). Ions in the air are in dynamic by the process of 'air ionization' and 'ionic recombination' caused by cosmic rays, radioactive substances and Lenard effect (Wang and Li, 2009).

The NAI help prevent disease and bolster human immune system. Breathing in air rich in NAI can improve human comfort index (Singh *et al*., 2009; Shepherd *et al*., 2010). The biological effect research on NAI by many scientists shows that it helps lower the temperature of rectums (Wakamura *et al*., 2004) and relieve severe stress of people who work indoors, especially computer workers (Krueger *et al*., 1974; Nakane *et al*., 2002). On the contrary, physical function could be disordered due to the surrounding operating environment with lack of NAI (Li, 2001). The NAI concentration varies greatly with the change of environment. For example, in a room with poor ventilation, the average concentration of NAI is as low as 300–500 ion·cm^{-3} (Liang *et al*., 2014; Wu *et al*., 2014a). Especially in polluted air, the average concentration even

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can be reduced to 0 ion·cm^{-3} (Tikhonov *et al*., 2004; Wang and Li, 2009), which is far below 1 500 ion·cm^{-3}; a standard average concentration in fresh air (Li, 1996). However, the NAI concentration is over 3 000 ion·cm^{-3} in different types of outdoor environments rich in vegetation, such as greenbelt, forest region, waterfall, park etc. (Iwama, 2004; Li *et al*., 2013).

Photosynthesis is one of the prime reasons, and air ionization caused by some blade shapes characteristics increase the concentration of NAI (Shao *et al*., 2005). Meanwhile, the temporal and spatial NAI concentration is greatly affected by environmental meteorological factors such as temperature, humidity, wind, particles in the air and radiation (Kinne, 1997; Li *et al*., 2013). In short, the concentration of NAI outdoor is much higher than that of indoor. Unfortunately, most people spend approximately 80–90% of 24 h in a whole day working or playing in indoors. In this period, they cannot enjoy the fresh air, which may enriched them in NAI from plants in natural conditions (Wu *et al*., 2011; Wu *et al*., 2014a). Therefore, it is of great importance to increase the concentration of NAI in indoor environments, especially in operating locations.

Some researches mainly focus on the NAI generation by phytocoenosium and its dynamic distribution features. Pan *et al*. (2013) pointed out that NAI concentration was dramatically different due to the structure and type of phytocoenosium, and that the structure of tree-shrub-grass was the highest one. NAI concentration was impacted by the combined effects of seasonal factors and community factors. In general, the concentrations of NAI in summer are greater than those in autumn. In addition, the leaf area index is positively correlated with NAI concentration (Xin *et al*., 2011). Skromulis and Noviks (2012) analyzed the correlation between the concentration of NAI and meteorological factors, with the highest level of NAI concentration measured in mornings.

Some scientists focus attention on the concentration of NAI in different vegetation such as urban green space and scenic spots as well as eco-efficiency evaluation of NAI (Pan and Dong, 2010; Ren *et al*., 2010). But there is little research on the generation of NAI by an individual plant. Wu *et al.* (2011) has reported research on the capacity of plants to generate NAI in a natural state. The results showed that the capacity of all plants to generate NAI in a natural state is weak. The NAI concentration released by an individual plant is no more than 300 ion·cm^{-3}. With such a concentration there is a limited biological and ecological effect. However, under the condition with high-voltage pulsed electric field (PEF) and light stimulation, plants generate a more considerable amount of NAI (Tikhonov *et al*., 2004; Wu *et al*., 2014b; Wu *et al*., 2015). It has been shown that it is due to physiological changes other than physical changes that make plants release NAI (Tikhonov *et al*., 2004). However, it is still unclear on the mechanism of NAI generation by plants under the stimulation with PEF and light is still unclear.

In this study we focused mainly on the effect of PEF and light stimulation on NAI generation by plants, and the correlation between the capacity of NAI generation and characteristics of plant stomata. It was hypothesized that the stomata is a main channel for generating NAI of plants, while the light intensity is one of significant parameters on producing NAI by plants. Thus, the objectives of this study were to explore the effects of stimulation of pulsed electric filed and light intensity on producing NAIs by plants and the relationship between NAI release and the stomatal characteristics under the stimulations of PEF. Our results lay a foundation for the study of NAI generation mechanism of plants and help find the ways to improve the capacity of plants to generate more NAI.

Materials and Methods

Materials

In order to study the influence of blade shapes and plant morphology on NAI generation of plants, we chose 10 species of plants (Table 1) including *Monstera adansonii* var. *laniata* (Schott) Madison (rohdea), *Crinum asiaticum* var. *sinicum* (Roxb. ex Herb.) Baker (poison bulb), *Ophiopogon bodinieri* Lévl. (ophiopogon), *Alocasia macrorrhizos* (L.) G. Don (alocasia), *Asparagus densiflorus* (Kunth) Jessop (lucid asparagus), *Dianella ensifolia* (Linn.) DC. (dianella), *Narcissus tazetta* var. *chinensis* Roem. (narcissus), *Hemerocallis fulva* (Linn.) Linn. (tawny daylily), *Zephyranthes carinata* Herb. (zephyranthes), and *Dracaena cochinchinensis* (Lour.) S. C. Chen (dracaena). All these plants were bought in the flower market in Baihua Village, Zhangzhou City, Fujian Province. Three plants with the same shape were selected from each species for three experiments. All the plants were planted in plastic flowerpots with outer caliber of 23 cm, height of 19 cm and width of 15 cm. They were given the same amount of water and fertilizer. The optimal NPK ratio put forward by Xia (2014) was adopted for once a week in order to ensure the normal growth of plants.

Measurement of NAI Concentration

The experiment was conducted on sunny days from June to October in 2016. Sunny days can ensure the similar light intensity in the indoor experiment, while avoiding experimental errors caused by light intensity. The measurement of NAI concentration was conducted in the sealed glass chamber with a size of 0.512 m^3 so as to exclude the influence of the already existing NAI concentration in indoors. We cut out a small opening with the size of 104×104 mm^2 on one of sides of the glass chamber. The size of the small opening was the same as the air inlet of DLY-4G-232 air ion counter. The opening was blocked with the baffle glass in order to avoid the effect of the NAI concentration of outside chamber (Fig. 1). The

Table 1: Tested plant species

Code	Plants	Age (month)	Plant height ×Grown breadth(cm)
P1	*Monstera adansonii* var. *laniata*	12	45*65
P2	*Crinum asiaticum* var. *sinicum*	24	42*53
P3	*Ophiopogon bodinieri*	13	13*15
P4	*Alocasia macrorrhizos*	13	70*43
P5	*Asparagus densiflorus*	12	25*30
P6	*Dianella ensifolia*	12	42*45
P7	*Narcissus tazetta* var. *chinensis*	36	30*22
P8	*Hemerocallis fulva*	12	46*32
P9	*Zephyranthes carinata*	12	41*42
P10	*Dracaena cochinchinensis*	24	45*53

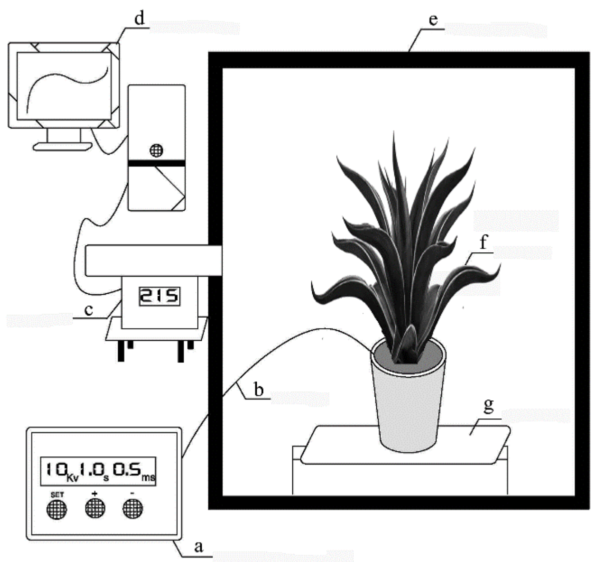

Fig. 1: Schematic of detecting negative air ions concentration by plant in a sealed chamber. a. High-voltage pulsed generator; b. Pulse probe: c. Air ions detector: d. Computer: e. Glass chamber: f. Plant: g. Adjustable insulation platform

center of the plant was adjusted to overlap the center of the glass chamber, so that the center of the air inlet of the air ion counter was at the same height with the top of the plant. The DLY-4G-232 air ion counter was produced by the Research Institute of Southeast Electronic Technology in Zhangzhou, China. The measurement range of NAI concentration is from 1 to 1.999 × 10^9 ion·cm^{-3}. The temperature ranged from 0–40ºC. The relative humidity was no more than 99%. And the measurements are transmitted through RS-232 linking port to the computer for information preservation. The air inlet of the air ion counter was connected with the small opening of the glass chamber. The air ion counter read data once per second and the sequential read access lasted for 150 s. The mean value of 150 sets of data was taken as NAI concentration for the individual plant. The mean value of the NAI concentration of three sets of data in three repeated experiments was taken as the NAI concentration of a plant species under the condition.

Measurement of NAI Concentration Released by Plants in Natural Condition

In natural condition, plants were put into the glass chamber for 15 min before the measurement of NAI concentration so as to exclude the influence of the existing NAI concentration in the glass chamber. The concentration of NAI was measured once per h for 24 times a day. The mean value of NAI concentration of each plant in each period was taken as NAI concentration released by this plant species. The mean value of NAI concentration in 24 periods was taken as the average concentration of NAI released from a plant species in the natural state. There were two control groups in the experiment. There was pot soil but without plants in one control group, while in the other control neither there was soil nor plants. The purpose was to exclude the influence of NAI of soil and air and had a more exact measurement of NAI concentration released by targeted plants in a normal state.

Measurement of NAI Concentration Released by Plants under PEF

The pulse generator developed by Fujian Agriculture and Forestry University was adopted in the experiment. The average output high-voltage pulse was U = 0.8×10^4–2.0×10^4 V; the pulse interval T = 0.5–2.0 s and the pulse width τ = 5–90 ms. The probe of the pulse generator was buried 5 cm deep in the soil and 5 cm from the periphery of the stem so that the negative high-voltage pulses are conducted to the soil through the probe. The method for data recording was the same as the method for measurement in a natural state. The mean value of the NAI concentration of three sets of data in three repeated experiments was taken as the NAI concentration released by this plant species in PEF.

To study the capacity of plants to release NAI when different intensities of PEF were applied, the orthogonal design experiment of three factors (pulse voltage A, pulse interval B, pulse width C) and four levels (Table 2). A total of sixteen treatment groups were set up. And there were three repeated experiments in each treatment groups to measure NAI concentration. A control group was also set up and there was only the pot soil in the control group. The purpose was to study the capacity of pot soil to generate NAI under different PEF, thus excluding the influence of pot soil on the generation of NAI by plants. Methods for measurement and data collection have been given in the previous part of measurement of NAI concentration.

Measurement of the Voltage of Plants with PEF

In order to explore the relationship between the voltage of plants and capacity of NAI generation, three experimental groups were set up, including the control group and experimental group A and experimental group B. Three repeated experiments were conducted in each experimental group with the optimal combined parameters of PEF. The

Table 2: Factors and levels for orthogonal test

Level	Factors		
	A, Pulsed Voltage (10^3 V)	B, Pulsed interval (s)	C, Pulsed Width (ms)
1	8	0.5	5
2	10	1.0	35
3	15	1.5	65
4	20	2.0	90

optimal parameters for plants to release NAI were obtained from the orthogonal design experiment in the previous part. The control group: the optimal parameters of PEF were applied to rhizosphere soil of the plant. The PINTEK HVP-40 high-voltage test pen was connected to the TDO1000/2000 digital storage oscilloscope. It read the voltage value of plants and at the same time detects NAI concentration generated by plants. The experimental group B: under the optimal combined parameters of PEF, NAI concentration released by the plant and the voltage of the plant were measured by using the wire to connect the plant with physical ground (the earth). The experimental group C: under the optimal parameters of PEF and NAI concentration released by the plant and the voltage of the plant were measured by using the wire to connect the plant with the ground wire.

NAI Generation by Plants with PEF and Light Intensity

For the sake of exploring the correlation between the capacity of plants to generate NAI and different light intensity under both conditions of the stimulation of PEF and normal, the experiment was carried out in Hipoint 740FHC light growth incubator, which is produced by Taiwan Hipoint Co., Ltd. The range of light intensity adjustment was from 0 to 100%. The Hipoint HR-350 spectrum analyzer produced by Taiwan Hipoint Co., Ltd. was used for the measurement and calibration of light intensity. The measurement range was from 0 to 100,000 lumen • m^{-2} and the measurement range of wavelength was from 360 to 760 nm. There were two experimental groups. The control group without the stimulation of PEF, while the stimulation group used the optimal combined parameters of PEF. Six treatment groups were set up in each experimental group, respectively with the intensity level of 0, 500, 1,500, 3,000, 6,000 and 12,000 lumen·m^{-2}. Three repeated experiments were conducted in six treatment groups to find out the correlation between the capacity of plants to generate NAI and different light intensity under PEF and normal state. Methods for NAI concentration measurement and data collection have been given in the previous part of measurement of NAI concentration.

Leaf Stomatal Characteristics Analysis

For the purpose of exploring the relationship between stomatal characteristics of leaf epidermis and the capacity of NAI generation by plants, two experimental groups were set up. One was the control group in which plants were grown in a natural condition, while the other was the experiment group with stimulated of optimal combined parameters of PEF applied to rhizosphere soil of plants. Each plant was sampled after 600 s of continuous stimulation and the concentration of NAI generated by plants was measured.

Using the method of Zhang *et al*. (2008) the selected sampling part was near 0.3 to 0.5 cm of the midribs of leaves, which was cut into a square with the area of 3 to 5 cm^2. The sample was fixed in formalin-acetic-alcohol (FAA) solution for 24 h, and then water-bathed at 60ºC for 40 min. After that, it was soaked in 30 to 40% of sodium hypochlorite for 24 h put in the distilled water the upper and the lower epidermis were peeled off. With 0.5% of fast green stained solution, a glass slide was made for making observation under OPTEC (DV320) optical microscope at 20X. The quantitative characteristics were analyzed from 10 points. When measuring the length, width and area of stomata, the microscope was at 40X. During the observation of quantitative characteristics of stomata, the mean average of 200 sets of data were taken, averaged according to calculate the ratio of stomatal length and width.

Statistical Analysis

For the purpose of presentation the treatments are designated as: A, Average output voltage (10^3 V); B, Pulse interval(s) and C, Pulse width (ms). Further treatment combinations are given in Table 4. All data processing procedures were processed by using Microsoft excel 2016. Experiment data were calculated and subjected to analysis of variance (ANOVA) and LSD using IBM SPSS Statistics (version 20.0, IBM Corporation, Armonk, New York and USA).

Results

Analysis of Daily Variation of NAI Concentration Generated by Plants in the Normal State

Fig. 2 shows that under natural conditions the average NAI concentration respectively released by ten plant species was very low during the day although it was quite smooth and steady. Particularly, for the NAI concentration produced by control group the minimum NAI concentration was 31 ion·cm^{-3} and the maximum 40 ion·cm^{-3} during those period. From the perspective of the extreme NAI concentration in a whole day, among these plants, the poison bulb released the maximum NAI concentration with 99 ion·cm^{-3} at 13:00 (Table 3). There was 2.5 times of the maximum NAI concentration in control groups, and over 2.6 times of that by pot soil. On the contrary, the rohdea generated the minimum NAI concentration with 26 ion·cm^{-3} at 22:00. The narcissus produced the maximum average NAI

Table 3: Analysis of negative air ions concentration generated by plants between 24h in natural conditions

Code	24 h minimum (ion·cm^{-3})	24 h maximum (ion·cm^{-3})	24 h mean (ion·cm^{-3})	Daytime mean (ion·cm^{-3})	Nighttime mean (ion·cm^{-3})	Max/Min	Day mean/ mean	(Max-min)/min	(Day mean-night mean)/night mean	Day mean/night mean
CK	31	40	34i	36	33	1.29	1.06	0.29	0.09	1.09
P0	32	38	35h	36	34	1.19	1.03	0.19	0.06	1.06
P1	26	46	36h	38	34	1.77	1.06	0.77	0.12	1.12
P2	53	99	68b	73	62	1.87	1.07	0.87	0.18	1.18
P3	33	79	56d	61	51	2.39	1.09	1.39	0.20	1.20
P4	54	81	66c	69	64	1.50	1.05	0.50	0.08	1.08
P5	46	69	55e	56	55	1.50	1.02	0.50	0.02	1.02
P6	30	49	40g	39	42	1.63	0.98	0.63	-0.07	0.93
P7	54	88	73a	74	72	1.63	1.01	0.63	0.03	1.03
P8	37	80	55d	62	49	2.16	1.13	1.16	0.27	1.27
P9	54	90	72a	68	76	1.67	0.94	0.67	-0.11	0.89
P10	35	53	43f	43	42	1.51	1.00	0.51	0.02	1.02

CK; P0 pot soil (without plant);P1 *M. adansonii* var. *laniata* ; P2 *C. asiaticum* var. *sinicum*; P3 *O. bodinieri*; P4 *A. macrorrhizos*; P5 *A. densiflorus*; P6 *D. ensifolia*; P7 *N. tazetta var. chinensis*; P8 *H. fulva*; P9 *Z. carinata*; P10 *D. cochinchinensis*. Different letters in each column indicate significant difference at P<0.05 (LSD test)

Table 4: Analysis of negative air ions concentration generated by plants upon different combinational parameters of pulsed electrical stimulation

Treatment	Factors			Negative air ions concentration (NAIC, ion·cm^{-3})			
	A	B	C	P0	P2	P7	P9
$A_1B_1C_1$	8	0.5	5	34±3a	5526±219de	85±3a	270156±32772j
$A_1B_2C_3$	8	1.0	65	33±2a	4717±219e	71±5a	319667±31589ij
$A_1B_3C_4$	8	1.5	90	34±2a	5353±250de	79±3a	279333±45319j
$A_1B_4C_2$	8	2.0	35	34±2a	3947±271e	89±5a	291800±27229ij
$A_2B_1C_4$	10	0.5	90	29±2a	157911±26785ab	85±9a	336667±24631hi
$A_2B_2C_2$	10	1.0	35	28±2a	161533±26643ab	78±8a	374556±23025h
$A_2B_3C_1$	10	1.5	5	26±3a	161733±25959ab	75±7a	320600±41121ij
$A_2B_4C_3$	10	2.0	65	34±3a	146367±27832b	81±4a	343356±54765hi
$A_3B_1C_2$	15	0.5	35	96±2a	195556±14960ab	126±20a	1486222±162985g
$A_3B_2C_4$	15	1.0	90	96±2a	201000±23798a	150±40a	1791067±272434e
$A_3B_3C_3$	15	1.5	65	100±3a	192311±21705ab	133±16a	1516667±252872g
$A_3B_4C_1$	15	2.0	5	97±2a	172133±19342ab	162±28a	1685133±281013f
$A_4B_1C_3$	20	0.5	65	125±2a	59475±839c	270±39a	3593489±358104a
$A_4B_2C_1$	20	1.0	5	123±5a	58917±962cd	279±18a	2979822±274258c
$A_4B_3C_2$	20	1.5	35	126±3a	48717±586cde	315±33a	3224000±299389b
$A_4B_4C_4$	20	2.0	90	131±4a	52917±698cde	264±51a	2860600±329636d

A: Average output voltage (10^3 V); B: Pulse interval(s); C: Pulse width (ms); P0: pot soil; P2 *C. asiaticum* var. *sinicum*; P7 *N. tazetta var. chinensis*; P9 *Z. carinata*. NAIC value indicate the mean ± SD. Different letters in each column indicate significant difference at P<0.05 (LSD test)

concentration with 73 ion·cm^{-3}. The average NAI concentrations of zephyranthes and poison bulb were 72 and 68 ion·cm^{-3} and therefore the capacity to generate NAI of them were ranked second and third respectively. Rohdea had the lowest ability to released NAI with the mean value of 36 ion·cm^{-3} among these ten plant species. Although the average NAI level released by ten plants vary slightly, there was a significant difference among NAI concentration generated by those plants (P<0.05). According to the mean value of NAI concentration both during the day (7:00 AM to 7:00 PM) and night (7:00 PM to 7:00 AM) time excluding the dianella and zephyranthes the average NAI concentrations of other plants during daytime were higher than those in nighttime (Table 3). Particularly, the daylily had the maximum growth and to compare with the average NAI concentration during the night, its capacity to generate NAI was increased by 27%.

The Optimal Parameters of PEF for Efficiently Generating NAI

The poison bulb, narcissus and zephyranthes had slightly stronger ability to generate NAI in a natural state chosen to carry out experiments. The range of NAI concentration ranged from 26 to 131 ion cm^{-3}. Although the plants produce different amounts of NAI concentration with different parameters of PEF there was no significant (P>0.05) difference of NAI concentration released by pot soil in different treatments (Table 4).

The poison bulb produced different amounts of NAI under different parameters of PEF. Its average NAI concentration ranged from 3,947 to 201,000 ion·cm^{-3} (Table 4). Under the parameters of $A_3B_2C_4$, the NAI concentration was the highest, which was 2 955.9 times that of average NAI concentration released in a natural state and 2 093.8 times that of the average NAI concentration of the pot soil

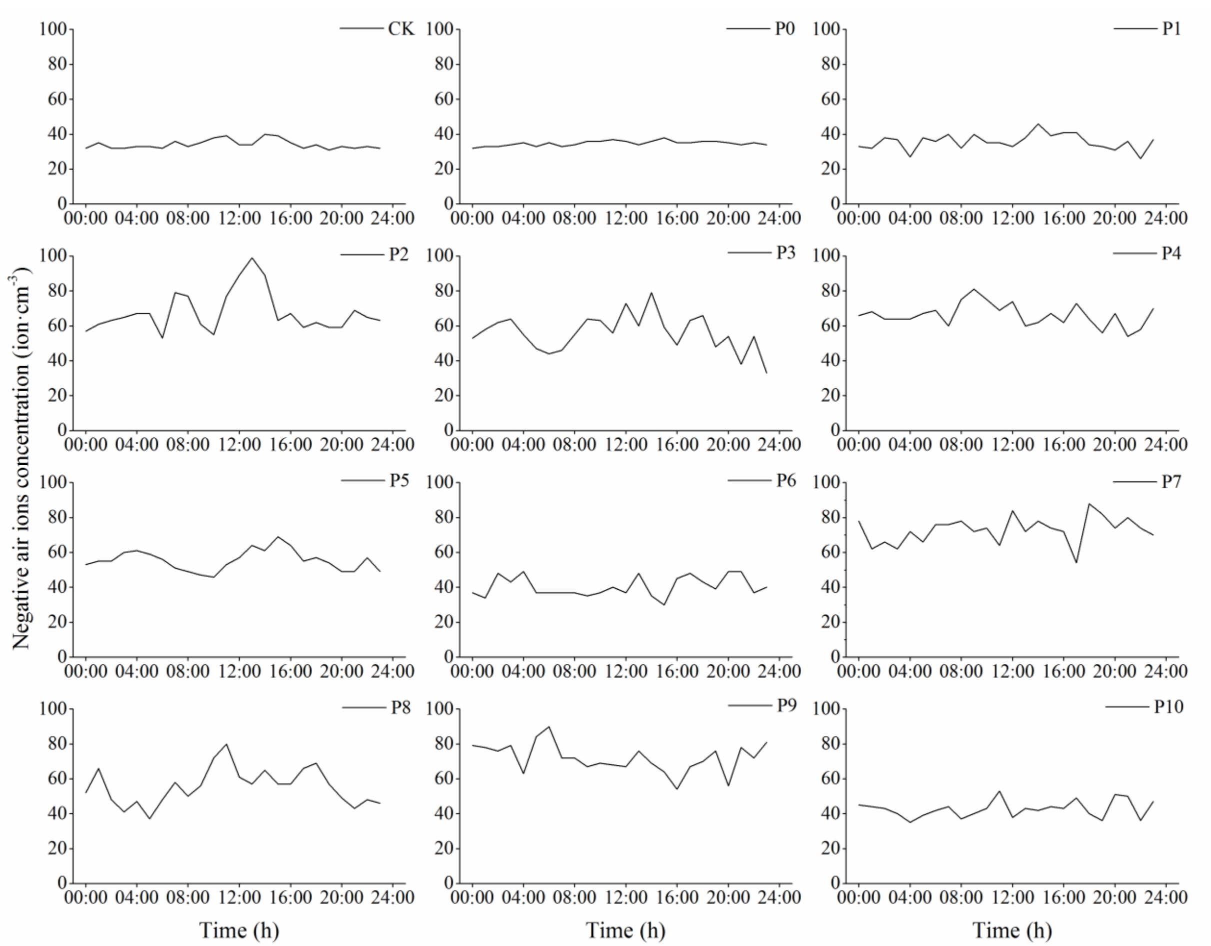

Fig. 2: The changes in negative air ions concentration over 24h for each of plant species under normal conditions. CK; P0 soil; P1 *M. adansonii* var. *laniata* ; P2 *C. asiaticum* var. *sinicum*; P3 *O. bodinieri*; P4 *A. macrorrhizos*; P5 *A. densiflorus*; P6 *D. ensifolia*; P7 *N. tazetta var. chinensis*; P8 *H. fulva*; P9 *Z. carinata*; P10 *D. cochinchinensis*

under electrostimulation with the same parameters. Under the parameters of $A_1B_4C_2$, the NAI concentration was the lowest with the average NAI concentration of 3 947 ion·cm^{-3}. It was 58.0 times of the NAI concentration in a normal condition and 116.1 times of the NAI concentration of the pot soil with the stimulation of the same parameters. The variance analysis showed that the optimal combined parameter for the poison bulb was $A_3B_2C_4$. Under the stimulation of these parameters of PEF the amount of NAI concentration released by the poison bulb increased the most.

With the stimulation of different combined parameters of PEF the average concentration of negative air ions generated by narcissus was from 71 to 315 ion·cm^{-3}. Among them, the parameters $A_4B_3C_2$ allowed narcissus to generate the highest amount of NAI with 315 ion·cm^{-3}. It was 4.3 times greater NAI generated in natural condition and 2.5 times the capacity to generate NAI with the same parameters applied to the soil. Narcissus generated the least NAI when parameters $A_1B_2C_3$ was applied. The NAI generation capacity was similar to that (73 ion·cm^{-3}) in natural condition and 2.2 times higher than the parameters $A_1B_2C_3$ applied to pot soil. Although narcissus generated different amounts of NAI with different combined parameters, there was no significant difference between those NAI concentrations under all different treatments (P>0.05). Variance analysis that among pulse voltage, pulse interval and pulse width, only pulse voltage has significant effect on the NAI generation of narcissus (P<0.05) and that the $A_4B_3C_2$ was the optimal combined parameters of PEF.

The range of NAI concentration of zephyranthes was from 270 and 156 ion·cm^{-3} to 3,593,489 ion·cm^{-3} in the simulation with different parameters of PEF (Table 4). Zephyranthes has the highest capacity to produce NAI under the stimulation of parameters $A_4B_3C_2$, which was 49,909.6 times greater than its capacity in natural state 28 747.9 times greater than the pot soil's ability to apply the same parameters. Its impact on generating NAI was dramatically different from other combined parameters (P<0.05). Under the $A_1B_1C_1$ zephyranthes generates the lowest NAI concentration of 270 and 156 ion·cm^{-3}, 3,752.2 times greater than the amounts of NAI in natural condition and 7,945.8 times greater than these with this parameters applied to the pot soil. Variance analysis showed that pulse voltage, pulse interval and pulse width all have remarkable effect on the concentration of NAI generating by zephyranthes (P<0.05). The $A_4B_1C_3$ was the optimal combined parameter for zephyranthes with 3,593,489 ion·cm^{-3} average concentration of NAI. Compared with the amounts of NAI that plants generate in natural conditions the concentration of NAI has been improved by different degrees when the stimulation with different combined parameters were applied. Except narcissus, both poison bulb and zephyranthes have significant difference in generating NAI when applied different combined parameters of PEF (P<0.05; Table 4).

Relation between NAI Generation and Plant Voltage

There was a positive correlation between the voltage across the plant body and the ability to generate NAI. The NAI concentration of poison bulb, narcissus and zephyranthes for control group was higher than those of the corresponding treatment group A and B of each plant (Table 5). Except the fact that narcissus has no significant difference between these NAI concentration in control group CK, group A and B, both poison bulb and zephyranthes have remarkable difference between the results of group A and B their CK (P<0.05). In control groups, compared with input voltage (the voltage of optimal PEF) the voltage of these three plants were all reduced by different degrees. Among them, the reduction of zephyranthes was the highest one, with the voltage of 3.64×10^3 V, decreasing 81.8% of the input voltage, and the NAI concentration was 3,613,489 ion·cm^{-3}. Compared with input voltage there was 77.5% reduction of the voltage of poison bulb and narcissus with 3.37×10^3 V and 4.50×10^3 V, respectively and their NAI concentration was 191 000 and 348 ion·cm^{-3}, respectively. Although narcissus has the highest voltage on plant body among three plants, its ability to generate NAI was the least (Table 5).

Considering the result of group A the reduction of the voltage of these three plants has significant difference between their counterparts in CK group (P<0.05). The plant voltage of poison bulb was reduced by 94.5% compared with the input voltage. In addition, compared with control group, its NAI concentration was reduced by 99.7%. For narcissus these two reduction figures were 94.2 and 62.1% respectively. And approximate situation in zephyranthes the reduction was 95.0 and 99.9% respectively. Under the condition of treatment B the voltage of the three plants further reduce and the ability of their NAI generation was nearly equal to that in natural state (Table 5).

Relation between NAI Generation and Light Intensity

Table 6 demonstrates that without electrostimulation the concentration of NAI of pot soil had the tiny variation ranging from 33 to 41 ion·cm^{-3}. The amounts of NAI generation of poison bulb, narcissus and zephyranthes increase slightly with more high light intensity. Although the absolute variation of NAI level was slight there existed remarkable differences in different light intensities (P<0.05; Table 6). The NAI concentration of poison bulb ranges from 48 to 128 ion·cm^{-3}, while narcissus was from 69 to 121 ion·cm^{-3}. Under the light intensity of 12,000 lumen·m^{-2}, the NAI concentration of zephyranthes was 132 ion·cm^{-3}, and it was 64 ion·cm^{-3} under darkness condition (0 lumen·m^{-2}).

When optimal PEF was applied, the amounts of NAI of pot soil and the three plants significantly were improved (P<0.05) compared with those of no stimulating conditions. There was no significant difference of the NAI concentration generated by pot soil and narcissus under different light intensities (P>0.05). When the light intensity was from 1,500 to 12,000 lumen·m^{-2}, the NAI concentration of poison bulb was from 211,000 to 225,356 ion·cm^{-3}, with no obvious difference in the capacity to generate NAI (P>0.05).When the light intensity was from 500 to 1 500 lumen·m^{-2} and from 3,000 to 6,000 lumen·m^{-2}, there was no significant difference in the ability of NAI generation of zephyranthes (P>0.05). But under the situation with other light groups the differences were dramatic (P>0.05). In general, when PEF was applied and with gradually increased light intensity the NAI concentration of three plants was improved at different levels; among which the increase of zephyranthes was the least. Its NAI concentration was doubled in the light intensity of 12 000 lumen·m^{-2} than that in darkness. Whereas the amounts of NAI generation of poison bulb and narcissus were 2.2 times greater than those in 0 lumen·m^{-2} light intensity. The NAI concentration of narcissus, however, was much lower and its maximum value was only 365 ion·cm^{-3}, far lower than the poison bulb's.

Relation between NAI Generation and the Epidermal Stomata

When optimal PEF was applied the stomata of poison bulb were open, as was nearly the similar to normal condition (Fig. 3A, B). There was a slight variation in the length and width of epidermal stomata. The length of stomata with electrostimulation was reduced by 0.4% compared with CK group, while its width increased by 10.6%. Although there was difference in the data, such difference was insignificant (P>0.05). Under stimulating conditions, the area and perimeter of stomata compared with CK group increased by 10.2 and 0.4% respectively both with no remarkable difference (P>0.05). The stomatal density compared with CK group increased 10.5% have significant difference (P < 0.05). Moreover the concentration of NAI of poison bulb was 3 112.7 times greater than that of CK group with the conspicuous difference them (P<0.05; Table 7).

Under the stimulation of optimal combined parameters

Table 5: Analysis between voltage of plants and negative air ions concentration (ion·cm^{-3})

Treatment	*Crinum asiaticum*		*Narcissus*		*Zephyranthes*	
	Voltage (10^3 V)	NAIC (ion·cm^{-3})	Voltage (10^3 V)	NAIC (ion·cm^{-3})	Voltage (10^3 V)	NAIC (ion·cm^{-3})
CK	3.37±0.11a	191000±23798a	4.50±0.15a	348±33a	3.64±0.13a	3613489±358104a
A	0.82±0.06b	594±29b	1.17±0.13b	132±11a	1.00±0.05b	2488±183b
B	0.24±0.04c	86±6b	0.40±0.02c	76±6a	0.43±0.02c	79±5b

CK (control group) refers to the condition when pulsed electrical field with optimal combinational parameters is applied to the rhizosphere soil (Crinum asiaticum: U = 15 × 10^3 V, t = 1.0 s, τ = 90 ms; Narcissus: U = 20 × 10^3 V, t = 1.5 s, τ = 35 ms; zephyranthes: U = 20 × 10^3 V, t = 0.5 s, τ = 65 ms). Treatment A refers to the condition where optimal combinational parameters of pulsed electrical field wrere applied to the plant, and a wire is used to connect the plant to the physical ground (earth). Treatment B refers to the condition where optimal combinational parameters of pulsed electrical field were applied to the plant, and a wire is used to connect the plant to the ground. Value indicate the mean ± SD. Different letters in each column indicate significant difference at *P*<0.05 (LSD test)

Table 6: The negative air ion concentration of plants under pulsed electrical field stimulation in different light intensity (ion·cm^{-3})

Light intensity (lx)	Soil		*Crinum asiaticum*		*Narcissus*		*Zephyranthes*	
	CK	S	CK	S	CK	S	CK	S
0	33±2c	124±3a	48±2f	98000±11995b	69±2e	169±16a	64±2e	2001356±200586d
500	34±2c	134±2a	67±3e	204600±20418ab	73±4d	326±32a	67±3d	3466333±347620c
1500	35±3bc	128±2a	69±2d	211000±23824a	76±3c	337±33a	75±2c	3573489±358502c
3000	35±3bc	132±2a	78±3c	220911±24144a	73±3d	352±34a	77±5c	3787978±379622b
6000	41±2a	138±2a	85±3b	218867±26942a	99±3b	349±34a	91±3b	3696867±385363b
12000	37±3b	130±3a	128±4a	225356±27751a	121±3a	365±35a	132±3a	3923756±393494a

CK: Without stimulation; S: Electrostimulation with optimal pulsed electrical field. Value indicate the mean ± SD. Different letters in each column indicate significant difference at *P*<0.05 (LSD test)

Table 7: The negative air ions concentration and stomata quantitative feature of plants under pulsed electrical filed stimulation

Plants	Treatment	Length (μm)	Width (μm)	Length/width	Perimeter (μm)	Area (μm^2)	Stomatal density (mm^{-2})	NAIC (ion·cm^{-3})
P2	CK	25.09±4.93a	3.12±0.99a	9.00±3.94a	53.73±9.83a	61.09±22.07a	50.72±7.81b	71±2b
	S	25.00±3.09a	3.45±0.84a	7.74±2.41a	53.94±6.07a	67.35±17.20a	56.04±11.40a	221000±23824a
P7	CK	22.19±3.03a	2.53±0.60a	9.31±2.82a	47.27±6.16a	44.23±12.40a	44.14±10.70a	71±3a
	S	20.20±2.84b	2.56±0.63a	8.44±2.66a	43.32±5.76b	40.63±11.59a	47.74±9.64a	384±29a
P9	CK	40.17±4.77a	3.01±0.41b	13.57±2.37a	83.77±9.58b	95.09±18.12a	41.14±4.66a	75±2b
	S	40.97±3.38a	6.29±0.78a	6.60±0.88b	89.12±7.10a	203.05±34.33b	40.22±4.06a	3593489±358502a

P2: *C. asiaticum* var. *Sinicum*: P7 *N. tazetta var. Chinensis*: P9 *Z. carinata*. CK: Without stimulation; S: Electrostimulation with optimal pulsed electrical field. Value indicate the mean ± SD. Different letters in each column indicate significant difference at *P*<0.05 (LSD test)

of PEF the change of the stomata shape of narcissus was quite obvious compared with the CK group (Fig. 3C, D). The length of the stomata, compared with the CK group was reduced by 9.0% having significant difference between them (*P*<0.05). Its width, however, has no remarkable difference compared with CK group with an increase of 1.2%. The area of stoma decreased by 8.1% after processing by the stimulation of PEF (Table 7; Fig. 3C, D) with insignificant difference between them (*P*>0.05). The stomatal density compared with that in normal condition had no remarkable difference (*P*>0.05). However, the concentration of NAI in narcissus was 5.4 times greater than that of CK group, and there was no conspicuous difference between them (*P*>0.05).

The change of the stomatal shape of zephyranthes was different from the shape of the CK group changed from close to opening while the optimal PEF was applied (Fig. 3E, F). Comparing with CK group there was 2.0% increase in length of the epidermal stomata in the stimulation condition, but the change has insignificant difference (*P*>0.05). Both the width and area of stomata increased 1.1 times with the electrostimulation compared with CK group all of them have significant difference (*P*<0.05). And the amount of NAI generation of zephyranthes was 47 913 times greater than that of CK group existing significant difference (*P*<0.05).

Discussion

The NAI, known as 'air vitamin', has aroused people's widespreadconcern for its active role in improving air condition and promoting human health. Currently, the research on NAI generation of plants mainly concentrates mainly on the areas of phytocoenosium and indoor individual plants. The paper mainly studied on the effects that PEF, light intensity and their combined action have on the NAI generation of individual plant. Under natural conditions plants generate low amounts of NAI. Among 10 plant species the amount of NAI generation of zephyranthes was the highest only 72 ion·cm^{-3}. Wu *et al.* (2011) had a research on plants that can generate NAI in natural conditions and found that in 68 plant species that the highest

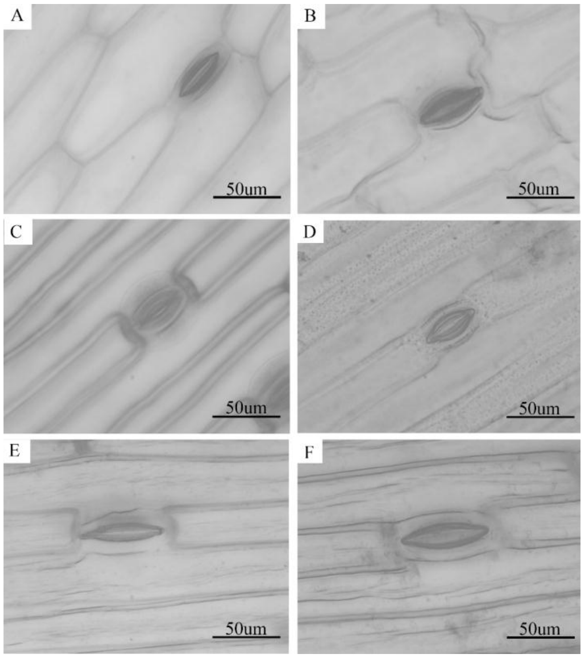

Fig. 3: The stomatal shape feature of three plant species under the stimulation of pulsed electrical field with optimal combinational parameters. The shape feature of three plant species were observed under 40× objectives. A–B The stomatal feature of *C. asiaticum* var. *sinicum* under normal and electrostimulation conditions, separately; C–D The stomata feature of *N. tazetta var. chinensis* under normal and electrostimulation conditions, separately; E–F The stomata feature of *Z. carinata* under normal and electrostimulation conditions, separately

average NAI concentration was only 73 ion·cm^{-3}, far lower than the standard concentration of 1500 ion·cm^{-3} of indoor living environment and has difficulty in conforming to the requirement of improving human health.

Plants however, generate much more amount of negative air ion when stimulated by PEF. Wu *et al*. (2015) applied PEF into rhizosphere of schlumbergera truncate and other plants finding that the increase range of plants NAI generation was from 843.1 to 48 498.9 times greater than that in natural conditions. Tikhonov *et al*. (2004) applied PEF on asparagus and found that the average NAI concentration increased from 133 ion·cm^{-3} (in normal conditions) to 12 5000 ion·cm^{-3}. Yang *et al*. (2009) also did the similar experiment and then confirmed the phenomenon. With the further experiment, our study found that different combined parameters of PEF can have different influences on NAI generation of plants. Then each plant species had the optimal combined parameter of PEF to generate high amounts of negative air ions respectively. The optimal combined parameter of poison bulb was $A_3B_2C_4$ (A_3，$U=1.5\times10^4$ V; B_2，T=1.0 s; C_4，τ =90 ms) with the NAI concentration of 201 000 ion·cm^{-3}, while the lowest combined parameter was $A_1B_4C_2$ (A_1，$U=0.8\times10^4$ V; B_4，T=2.0 s; C_2，τ =35 ms) with the NAI concentration of 3 947 ion·cm^{-3}. The optimal combined parameter of narcissus was $A_4B_3C_2$ (A_4, $U=2.0\times10^4$ V; B_3，T=1.5 s; C_2，τ =35 ms) with the NAI concentration of 315 ion·cm^{-3}; the lowest one was $A_1B_2C_3$ (A_1，$U=0.8\times10^4$ V; B_2，T=1.0 s; C_3，τ =65 ms) with the concentration of 71 ion·cm^{-3}. The optimal combined parameter of zephyranthes was $A_4B_1C_3$（A_4，$U=2.0\times10^4$V; B_1，T=0.5s; C_3，τ =35 ms) with the NAI concentration of 3593489 ion·cm^{-3}; the lowest one was $A_1B_1C_1$ (A_1，$U=0.8\times10^4$ V; B_1，T=0.5 s; C_1，τ =5 ms) with the concentration of 270 156 ion·cm^{-3}.

Different combined parameters of PEF produced different effects on NAI generation of plants. Yang *et al*. (2011) proposed that on one hand, the ability of NAI generation was significantly improved and that the electric field was applied to the plant should be sufficiently strong. On the other hand, if the electric field was too strong irreparable damage of plant cell may be caused. The conclusion of our study also confirmed this hypothesis to some extent. Under an electrostimulation of the optimal combined parameters of PEF the capacity of NAI generation by plant was the strongest. When the intensity of PEF was lower or higher than that of the optimal combined parameters, this ability will be decreased in different levels. The finding demonstrates that each plant has the optimal combined parameters to promote efficient NAI generation respectively. The reason may lie in the fact that biological effect under low-frequency electric field had 'threshold' and 'power window (Xi and Yang, 2008). Plants under this moderate stimulation can grow well and significantly can improve the ability of NAI generation without cell injury.

It was also found that the variation of average NAI concentration of narcissus was a lower when different intensities of PEF were applied into rhizosphere. Although with the rise of pulse voltage, there was a slight increase in the capacity of NAI generation of narcissus with the NAI concentration varying from 71 to 315 ion·cm^{-3}. The results of all the combined parameters had no remarkable differences due to the fact that plants have the specific frequency of potential characteristics the suitable stimulation of PEF is applied. This led to the resonance of electric field of plants, which improved plant's ability to generate NAI. As to the slight variation of NAI concentration of narcissus under all different intensities electrostimulation, it was supposed that the frequency of PEF does not match with the frequency of the plants (Xi and Yang, 2008). Therefore, no resonance happened between the PEF and the plant's electric field and the increase of NAI generation was reduced.

When the optimal combined parameters of PEF were applied to the three plant species the voltage and the NAI concentration of the plants were monitored. The result showed that there was a positive correlation between the voltage value of plant and its ability to generate NAI. For the same plant species with higher the voltage the capacity of NAI production was greater. In zephyranthes for an example, when the voltage across it was 3.64×10^3 V, the average NAI concentration was 3 613 489 ion·cm^{-3}. When the voltage was reduced to 0.43×10^3 V the amount of NAI was nearly equal to that in natural conditions (79 ion·cm^{-3}). It was supposed that the voltage stored in the plant body was analogous to the 'energy' supporting to the plant (Xi and Yang, 2008; Yang *et al*., 2011). The 'energy' may be considered to be the 'threshold' or 'power' which can arouse biological effects of generating NAI by plants. This represented that the pulsed voltage was just about a necessity "power" to open the passage for producing NAI by plants.

Light can also improve the ability to generate NAI of plants. Light stimulation was one of the main factors that had an influence on NAI generation of phytocoenosium. Wang and Li (2009) stimulated asparagus and other plants with light. Among these plants asparagus has the strongest response to light intensity. When the light intensity increased from 0 to 10,000 lumen·m^{-2}, the range of average NAI concentration was from 338 to 1,551 ion·cm^{-3}. In this paper, poison bulb, narcissus and zephyranthes were all insensitive to light intensity. The capacity of NAI generation of these plant species slightly increased with the higher light intensity and absolute variation was too small. This was due to the fact that plant species had different physiological characteristics and had a discrepancy of capacity to generate NAI between these plant species and the various impacts derive form light stimulation.

When the optimal combined parameters of PEF were accordingly applied to the three plants the average NAI concentration under different light intensities increased sharply compared with that in natural conditions. Among these plants the greatest ability of NAI generation was noted in zephyranthes, where NAI concentration under the stimulation of 0 lumen·m^{-2} light intensity was 2 001 356 ion·cm^{-3} when PEF was applied. This value was 31,271.2 times greater than that (64 ion·cm^{-3}) with the absence of PEF. Under the light intensity of 12,000 lumen·m^{-2} stimulation, the average NAI concentration of zephyranthes was 3,923,756 ion·cm^{-3} with PEF applied, while its average NAI concentration was 132 ion·cm^{-3} without PEF. The amount of NAI produced in zephyranthes was improved by 96.1%, when optimal PEF applied and the light intensity increasing from 0 to 12 000 lumen·m^{-2}. Possibly the light may stimulate the photoelectric effect of the leaf surface and cause the escape of free ions which later generate NAI. Meanwhile, it was found that under the optimal combined parameters the NAI generation of the three plant species has no significant difference, when the light intensity was from 3 000 to 6,000 lumen·m^{-2}. Zephyranthes was the most obvious one. When the light intensity was less than 3,000 lumen·m^{-2} or more than 6 000 lumen·m^{-2} the NAI concentration indicated remarkable difference. It was, therefore, concluded that the 'energy' provided by the light intensity, may be at the same level. Compared with 3 000 lumen·m^{-2} of light intensity, 6,000 lumen·m^{-2} cannot stimulate the further ability of NAI generation by plants.

Under PEF, the opening degree of leaf stomata of plants was different from that in natural conditions. Except narcissus, the stomatal area of the poison bulb and zephyranthes was much larger, when stimulated by PEF compared with the CK's. The capacity to generate NAI also improved with the expansion of stomatal area. For example in zephyranthes, before the PEF was applied, its stomatal area was 95.09 µm^2. After the application, the area was 203.05 µm^2 and the ability of NAI generation improved 47,912.2 times. Besides, the density of stomata was one of the key factors influencing the NAI generation in plants. In poison bulb, after the PEF was applied, the density of stomata improved 9.5% and the NAI concentration raised by 3 111.7 times compared with that in normal conditions. With an increase in the size and density of stomata the ability of NAI production improved sharply. This was possibly due to the reason that the stomata of leaf was a main channel for NAI generation. Zhang *et al*. (2016) reported that the density of thorns and stomata were the sites of NAI generation. Xi and Yang (2008) proposed a hypothesis that plants generate NAI by the electric discharge from stomatal transpiration, which was substantiated from our experiment. The stomata may discharge electricity through transpiration and the anion gathered on the leaves can escape by stoma transpiration and generate NAI.

It was further recorded that the opening degree of the leaf stomata of narcissus, on the contrary, was lowered when the PEF was applied. With improved NAI concentration the opening degree and NAI concentration was not different from the CK group. This is plausible that different plants have different tolerance towards PEF and also have different stomatal pore apparatus. The applied PEF may exceed the threshold of the 'power' that the narcissus needed to generate NAI causing injury of the plant (Xi and Yang, 2008; Wang and Li, 2009; Yang *et al*., 2009). The opening degree of the stomata, therefore, decreased and the channel to generate NAI could not completely open. So how to improve the opening degree of the stoma of plants has become an important factor for the improvement of NAI generation.

Conclusion

Under the optimal combined parameters of PEF, the capacity of NAI generation by plant also improved with the increased light intensity. There was a positive correlation between the voltage value of the plant and its ability of NAI generation. The opening degree and the density of the leaf stomata also had an impact on NAI generation of plants. Apart from PEF and light intensity, the NAI generation of plants was also influenced by other external factors (temperature, humidity and barometric pressure, etc.).

Certainly, the features of the plant itself were also important.

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