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Full Length Article

Stomatal Characteristics Determine the Negative Air Ions Produced by Plants under Pulsed Electrical Field

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Abstract

Naturally, plants possess a weak capacity of generating negative air ions (NAIs). NAIs were demonstrated as an indicator of air condition. Improving concentration of NAIs could be beneficial for human health. Previous studies revealed that stimulating plant by a pulsed electrical field (PEF) resulted in a burst of NAIs emission. However, little is known about the processing or mechanism of NAIs generation in plants under PEF stimulation. This study investigated the physiological mechanism underlying plant NAIs generation following PEF stimulation. We found that aloe (*Aloe vera* var. *chinensis* (Haw.) Berg.), sansevieria (*Sansevieria trifasciata* Prain) and agave (*Agave americana* var. *variegata* Linn.) naturally generated more NAIs than others. Various parameters (pulsed voltage, interval and width) of PEF input influenced the capacity of NAIs generation in plants. Optimal combinational parameters (CP) on of PEF for efficient generation of NAIs had species difference. Under optimal CP, a higher voltage of a plant was helpful for NAIs generation ($P < 0.05$). The intensity of illumination enhanced the ability of NAIs emission by plants ($P < 0.05$) under optimal CP stimulation. Furthermore, the capacity of NAIs generation by plants is highly related to the characteristics of leaf stomata. A greater degree of stomatal opening and stomatal density significantly enhanced the ability of NAIs emission from plants ($P < 0.05$). These results indicated that the presence of PEF stimulation, illumination intensity, plant voltage, and the specific plant physiological characteristics (e.g. stomatal area and stomatal density) influence plant NAIs generation. Our findings provide guidance for designing plant NAIs generator to improve air quality for human. © 2019 Friends Science Publishers

Keywords: Negative air ion; Electrostimulation; Pulsed electrical field; Light intensity; Stomata

Introduction

Negative air ions (NAIs) have been listed as an important indicator of air quality. The mainly existing form of negatively charged species of NAIs is $O_2^-(H_2O)_n$ (Goldstein *et al*., 1992; Kosenko *et al*., 1997; Liang *et al*., 2014). NAIs have been considered useful for disease prevention and enhancement of human and animal immune system (Singh *et al*., 2009; Shepherd *et al*., 2010).

NAIs were detected by DLY-4G-232 air ion counter. Different environments have significant difference of Negative air ions concentration (NAIC) (Horrak *et al*., 2003; Vana *et al*., 2008). In an enclosed room, NAIC may be as low as 300–600 ions·cm^{-3} (Ling *et al*., 2010; Liang *et al*., 2014). In particular, NAIC of a polluted air may drop to zero (Tikhonov *et al*., 2004). Importantly, NAIC can reach 0.3–1.9×10^{4} ions·cm^{-3} in environments with abundant vegetation (Iwama, 2004; Laakso *et al*., 2007; Wu *et al*., 2011a; Li *et al*., 2013; Liang *et al*., 2014). The variation of NAIC in the air is closely related to meteorological factors, such as the air temperature, the relative humidity, wind, and so on (Kinne, 1997; Shao *et al*., 2005; Li *et al*., 2013). Collectively, the concentration of NAI outdoors is much higher than that indoors (Hirsikko *et al*., 2007; Li *et al*., 2013). However, human activities are mostly occurring indoor, which results in lacking of NAIs source for sucking up (Wu *et al*., 2011b; Wu *et al*., 2014). Therefore, it would be important and meaningful to increase indoor NAIC to improve the air quality for human health.

It is known that plants play a key role in maintaining NAIC outdoors by release of NAIs (Wu *et al*., 2011a). Studies have revealed that stimulating plant by a pulsed electrical field (PEF) resulted in a burst of NAIs emission (Wu *et al*., 2017). By adopting this technology, we explored a device for significantly increasing indoor NAIC (Wu *et al*., 2017). However, little was known for understanding how did

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plant response to PEF stimulation and substantially increase surrounding NAIC.

It was reported that light intensity could significantly affect NAIC, which indicated that rate of plant photosynthesis might be attributed to the differences of NAIC in various areas (Wang and Li, 2009). In addition, leaf shape can lead to air ionization, which results in increasing NAIC (Tikhonov *et al.*, 2004). The aims of this study were to explore (1) the effects of stimulation of pulsed electrical filed and light intensity on the generation of NAIs by plants indoors; (2) the relationship between NAIs production and the plant's physiological characteristics (e.g. stomatal area and stomatal density) under the stimulations of pulsed electrical filed.

Materials and Methods

Materials

Aloe (*Aloe vera* var. *Chinensis* (Haw.) Berg.), sansevieria (*Sansevieria trifasciata* Prain) and agave (*Agave americana* var. *variegata* Linn.) with high NAIs emission under natural condition were selected for this study (Table 1). Plants were purchased from the flower market. Three individuals of each plant with similar shape/size were used as a replicate, and was planted in a plastic pot (top diameter: 23 cm, height: 19 cm, bottom diameter: 15 cm) indoor.

Measurement of Negative Air Ions Concentrations

The experiment was conducted from June to August 2015 on sunny days to maintain the approximate environment factors indoor during the experiments. A DLY-4G-232 Air Ion Detector was used to measure NAIC. The measuring range of the detector was 1–1.999 × 10^9 ions·cm^{-3}, and the operating temperature and humidity were 0–40°C and ≤ 90% RH. The data was transferred into a computer via RS-232 interface to analyze the NAI concentration. An 800 × 800 × 800 mm glass chamber made of organic glass material with a 4-mm thickness to minimize electrostatic properties was used to detect NAIC under the condition of preventing influences from environment factors accurately. On one side of the glass chamber, a small window of 104 mm × 104 mm, fitting the size of the measurement air inlet of DLY-4G-232 Air Ion Detector was accommodated. When no measurements were taken, this window was closed with a glass plate to avoid fresh air outside into chamber (Fig. 1).

The center of the potted plant was adjusted so that it overlaps the center of the chamber to keep the center of the air ion detector inlet at the same level with the top of the plant. The air ion detector reads data at every second, and the continuous effective reading time was 150 s. The mean of the data collected from 150 s of reading time was used as the NAIC of each individual plant. The mean value of three repetitions was considered as the capacity of generating NAI by that particular corresponding plant variety under the specified condition.

Measurement of Optimal Parameters of PEF

For electric stimulation of plant, we used the high voltage electrical pulse generator invented by Fujian Agriculture and Forestry University. The generator produced high voltage electrical pulses with the following parameters. The range of mean pulsed voltage (U) is from 8 × 10^3 V to 20 × 10^3 V, pulse interval (T) range from 0.5 s to 2.0 s, and pulse width range (τ) from 5 to 90 ms. Pulsed electrical field (PEF) stimulation was applied by inserting electrodes into rhizosphere soil of each pot. The negative pulse generator should be buried in the soil near the plant and exposed to the probe. The buried depth was 5 cm, approximately 5 cm away from the stem of the plant. NAIC surrounding plants under nature or with PEF stimulation were measured according to Wu *et al.* (2017). To explore the capacity of NAIs generation of plants under different PEF stimulation, a three-factor (average pulsed voltage A, pulse interval B, and pulse width C), four-level orthogonal design was applied (Table 2).

There were a total of 16 treatments, and for each treatment, with three repeats. One control of pot soil (without plant, P0) was used to detect ability of pot soil to generate NAI in absence of plants. Data acquisition and collection methods of all the experiments were the same as those mentioned in section of measurement of NAICs.

Measuring Voltage on Plants under Pulsed Electrical Field

To measure the voltage on plants, for each plant variety, one control group (CK) and two treatment groups (TGA and TGB) were prepared, and three replicates were set up for each group. PEF stimulation with optimal parameters was applied to the plants to explore the relationship between capacity of NAI generation and the voltage on plants. In control group (CK), optimal PEF stimulation was applied to the rhizosphere soil, and a PINTEK HVP-40 High Voltage Probe connected to a TDO1000/2000 Digital Storage Oscilloscope was used to measure plant voltage, the NAIC was measured simultaneously. In TGA, under optimal PEF stimulation, a wire was used to connect the plant to the physical ground (earth), and the voltage on the plant as well as the NAIC were measured. In TGB, a wire was used to connect the plant to the ground, and the voltage on the plant, as well as the NAIC, was measured (Wu *et al.,* 2017).

Measuring NAIs under Pulsed Electrical Field with a different Light Intensity

NAIs generation by plants is also affected by plant light intensity, as well as PEF. To explore the capacity of NAI generation affected by the interactions of PEF and light intensity, further experiments were carried out by combing the optimum PEF obtained from the above experiments and different light intensities (i.e. 0; 9; 27; 54; 108 and 216 μmol·m^{-2}·s^{-1}). This experiment was conducted in a Hipoint

740FHC Plant Growth Chamber, a Hipoint HR-350 Spectrum Analyzer was used for measurement and calibration of light intensity.

Two groups of each plant variety were set up in this experiment. One was the control group (CK), in which under the stimulation of illumination and without PEF. The other was treatment group, in which under the stimulation of combination of different light intensities and PEF with optimal parameters. Each control and treatment group consisted of three repeats. The effects of different illumination levels on plant NAI release under optimal PEF stimulation were examined. Data acquisition and collection methods of all the experiments were the same as those mentioned in section of measurement of NAICs.

Leaf Stomata

Leaf samples were collected from CK plants under normal condition or plants after 600 s of continuous optimal PEF stimulation. The NAICs of both were also recorded. According to Zhang *et al.* (2008), 0.3–0.5 cm away from the midrib and both sides of the leaf were collected for this study. Collected samples were cut into squares of 3–5 cm^2, and fixed in formalin-acetic-alcohol (FAA) solution for 24 h, then incubated for 40 min in a 60.0°C water bath. After that, the materials were soaked in 30%–40% sodium hypochlorite for 24 h, and then transferred to distilled water. Leaf epidermis were stained with 0.5% fast green, and then mounted on a glass slide. Slides were examined under a microscope [OPTEC (DV320)]. Ten fields within regions between veins of leaf epidermis were examined, and number of stomata per field was counted. An objective lens with 20X magnification was employed in counting the number of stomata; for the measurement of the stomatal length, width, and area, an objective lens with 40× amplification was employed. To determine the length and width of stomata more accurately, about 200 measurements were used in calculating for the mean, as well as 200 measurements of the area and length-to-width ratio of stomata were used in calculating the mean.

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 3. 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

Optimal Parameters of Pulsed Electrical Field for NAI Generation by Plants

NAIC measurements revealed that aloe, sansevieria, and agave possessed relatively higher NAI generation capabilities than others in the natural condition. Hence, they were used to study the effects different combinational parameter (CP) of PEF on NAIs of plants (Table 3). Under the stimulation of PEF with CP $A_4B_1C_3$, the NAIC released by aloe reached the maximum value, which was 14,941.8-fold higher than that of soil and significantly differed from other CPs ($P < 0.05$). ANOVA showed that the optimal CP was $A_3B_1C_4$ (1,335,315 ion·cm^{-3}).

NAIC of sansevieria varied from 15 ion·cm^{-3} to 556 ion·cm^{-3} under different CPs. $A_4B_1C_3$ resulted in the highest NAIC of sansevieria, which was 23.2-fold higher than that of soil. No significant differences were found between different CP comparisons ($P > 0.05$). However, ANOVA showed that pulse voltage exerted a significant impact on NAIs release for sansevieria ($P < 0.05$).

Under different CPs stimulation, the mean of NAIs generated by agave varied from 26,544 ion·cm^{-3} to 2,967,444 ion·cm^{-3}. $A_3B_1C_2$ was the best CP for agave,

Table 1: Tested plant species

Code	Plant species	Plant age (month)	Plant height × crown breadth (cm)
P1	*Aloe vera* var. *chinensis*	24	15 × 8
P2	*Sansevieria trifasciata*	12	30 × 20
P3	*Agave Americana* var. *variegata*	14	30 × 35

Table 2: Factors and levels for orthogonal test

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

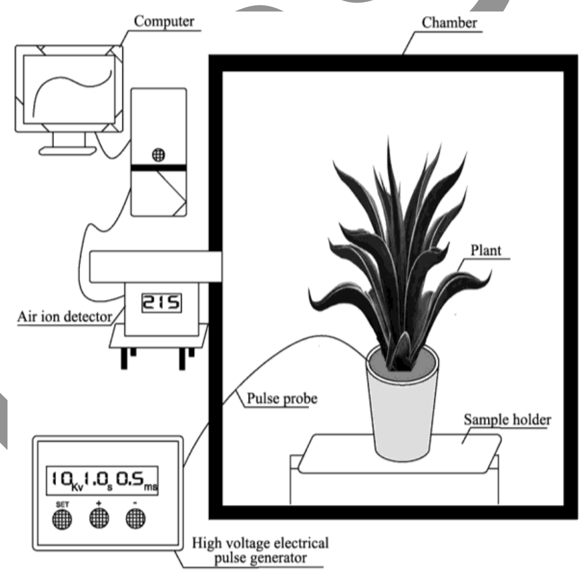

Fig. 1: Schematic of detecting the concentration of negative air ions by plant in a glass chamber

Table 3: Analysis of negative air ions concentration generated by plants under the stimulation of pulsed electrical field with various combinational parameters

Treatment	Factors			Average of negative air ions concentration (ion·cm^{-3})			
	A	B	C	Soil	Aloe	Sansevieria	Agave
$A_1B_1C_1$	8	0.5	5	28 ± 3Ab	552200 ± 22416Fa	15 ± 3Ab	34314 ± 4147Kb
$A_1B_2C_3$	8	1.0	65	28 ± 2Ab	470933 ± 20656Ga	51 ± 5Ab	36112 ± 3585Kb
$A_1B_3C_4$	8	1.5	90	29 ± 2Ab	534978 ± 25151Fa	49 ± 3Ab	26544 ± 4285Kb
$A_1B_4C_2$	8	2.0	35	29 ± 2Ab	393222 ± 22844Ha	29 ± 5Ab	32923 ± 3099Kb
$A_2B_1C_4$	10	0.5	90	24 ± 1Ac	318489 ± 52734Ib	161 ± 9Ac	1178778 ± 85721Ea
$A_2B_2C_2$	10	1.0	35	23 ± 2Ac	119644 ± 29618Kb	117 ± 8Ac	823622 ± 49392Ga
$A_2B_3C_1$	10	1.5	5	26 ± 3Ac	125422 ± 10085Kb	121 ± 7Ac	685911 ± 110613Ia
$A_2B_4C_3$	10	2.0	65	23 ± 3Ac	77878 ± 44673IKb	128 ± 4Ac	532833 ± 3113Ja
$A_3B_1C_2$	15	0.5	35	87 ± 2Ac	1347467 ± 83909Bb	448 ± 20Ac	2967444 ± 325295Aa
$A_3B_2C_4$	15	1.0	90	89 ± 2Ac	1291733 ± 96169Cb	458 ± 40Ac	2219044 ± 575630Ba
$A_3B_3C_3$	15	1.5	65	92 ± 3Ac	271733 ± 58565IJb	233 ± 16Ac	1504678 ± 4439Da
$A_3B_4C_1$	15	2.0	5	91 ± 2Ac	643111 ± 147442Eb	338 ± 28Ac	751000 ± 52150Ha
$A_4B_1C_3$	20	0.5	65	118 ± 2Ac	1763133 ± 153051Ab	556 ± 39Ac	1891022 ± 188510Ca
$A_4B_2C_1$	20	1.0	5	115 ± 5Ac	396800 ± 46989Hb	229 ± 18Ac	950844 ± 31279Fa
$A_4B_3C_2$	20	1.5	35	117 ± 3Ac	253978 ± 63498Jb	355 ± 33Ac	749644 ± 69681Ha
$A_4B_4C_4$	20	2.0	90	120 ± 4Ac	812778 ± 203361Db	364 ± 51Ac	733311 ± 84482HIa

Note: A: Pulse voltage (10^3 V); B: Pulse interval (s); C: Pulse width (ms); Different uppercase letters indicate significant differences within the same variety using pulsed electrical fields with different conditions, and different lowercase letters indicate significant differences ($P < 0.05$) among plant species using pulsed electrical fields

which significantly produced NAIs 33,342.1-fold higher than that of soil and 46,366.3-fold higher than that in the natural condition ($P < 0.05$). Pulse voltage also showed a significant effect on the NAIC generated by agave ($P < 0.01$).

The Relationship between NAI Generation and Voltage on Plants

For each plant, the voltage correlated with its NAI generation capacity. For all tested plants, the NAIC of CK was higher than that of two treatment groups (Table 4). Voltage on CK plant was attenuated to various degrees compared to the input voltage (pulsed voltage of the optimal PEF). The smallest attenuation was observed in aloe. Voltage on aloe was 2.35×10^3 V, which was reduced by 88.3% compared to the input voltage, and the NAIC was 1,737,244 ion·cm^{-3}. Agave appeared a second strongest attenuation with 1.60×10^3 V on plant. For sansevieria, although the voltage on the plant was closed to that of the aloe, NAIC was dramatically lower (Table 4). Based on treatment A, both voltage and NAIC on all three species were significantly lower than CK ($P < 0.05$). Moreover, the voltage on all three plants was reduced, and their capacities of generating NAI were close to normal level under natural conditions in treatment B (Table 4).

The Relationship between NAI Generation and Illumination

Without applied PEF, the capacity of generating NAI of soil and three plant species significantly differed with light of various intensities ($P < 0.05$). With higher illumination, mean of NAIC of all species increased gradually, but their magnitudes increased less. Agave produced a highest NAIC (127 ion·cm^{-3}) at illumination 216 μmol·m^{-2}·s^{-1}. Except for the soil and sansevieria, NAIC of plants significantly differed with various illumination levels under PEF stimulation ($P < 0.05$) (Table 5). Light intensity of 54 μmol·m^{-2}·s^{-1} and 108 μmol·m^{-2}·s^{-1} did not significantly differ the NAIC of three species ($P > 0.05$, Table 5).

Overall, with the increase of illumination, the NAIC of the three plant species showed an increase of varying magnitudes. Under the strongest illumination, the NAIC of agave and aloe were 2.3-fold and 1.9-fold respectively higher than that in the dark. NAIC of sansevieria did not show a significant difference under different levels of illumination ($P > 0.05$).

The Relationship between NAIC and Stomata

For aloe, the morphology of its stomata under PEF stimulation with optimal combinational parameters (CP) ($U = 20 \times 10^3$ V, $t = 0.5$ s, $\tau = 65$ ms) was similar to CK. In both cases, the stomata were almost closed (Fig. 2 A B). The length of stomata under optimal PEF increased ($P < 0.05$) by 27% as compared to CK (Table 6). However, the stomatal width did not significantly change ($P > 0.05$). The stomatal area under optimal PEF significantly increased ($P < 0.05$) by 29% when compared to CK (Table 6). There was no significant change in stomatal density. NAIC was significantly ($P < 0.05$) increased 27,886.1-fold under optimal CP stimulation when compared to CK (Table 6).

Optimal CP stimulation resulted in stomata closure of sansevieria (Fig. 2C 2D). Compared to CK, the length and width of the stomata on the leaf epidermis significantly decreased ($P < 0.05$). Stomatal area was also decreased by 72% with a statistically significant ($P < 0.05$) effect (Table 6). This stimulation did not affect stomatal density of sansevieria. NAIC of sansevieria under optimal PEF was 10.7-fold higher than that observed in CK. However, this difference was not significant ($P > 0.05$) statistically (Table 6).

Table 4: Analysis between voltage on plant species and negative air ion concentration (ion·cm^{-3})

Treatment	Aloe		Sansevieria		Agave	
	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	2.35 ± 0.09a	1737244 ± 122030 a	2.07 ± 0.1a	532 ± 9 a	1.6 ± 0.06a	2966044 ± 263954 a
TGA	0.44 ± 0.01b	468 ± 4 b	0.64 ± 0.01b	39 ± 2 a	0.64 ± 0.01b	178 ± 3 b
TGB	0.12 ± 0.02b	51 ± 6 b	0.16 ± 0.01b	32 ± 3 a	0.21 ± 0.01b	66 ± 5 b

Note: CK (control group) refers to the condition when pulsed electrical field (PEF) with optimal combinational parameters (CP) is applied to the rhizosphere soil (Aloe: U = 20 × 10^3 V, t = 0.5 s, τ = 65 ms; Sansevieria,: U = 20 × 10^3 V, t = 0.5 s, τ = 65 ms; Agave: U = 15 × 10^3 V, t = 0.5 s, τ = 35 ms); TGA refers to the condition where optimal CP of PEF are applied to the plant, and a wire is used to connect the plant to the physical ground (earth); TGB refers to the condition where optimal CP of PEF are applied to the plant, and a wire is used to connect the plant to the ground. Different lowercase letters denote significant differences (P< 0.05) among various treatments in the same cultivar

Table 5: Negative air ion concentration of plant species under pulsed electrical field stimulation and different light intensities

Light intensity	Soil		Aloe		Sansevieria		Agave	
(μmol·m^{-2}·s^{-1})	CK	S	CK	S	CK	S	CK	S
0	23 ± 2e	113 ± 3a	28 ± 2f	822356 ± 70769e	29 ± 2f	278 ± 20a	34 ± 2f	1670311 ± 175236e
9	24 ± 2d	123 ± 5a	64 ± 3e	1719800 ± 148054d	50 ± 4e	536 ± 38a	65 ± 3e	2893111 ± 303549d
27	25 ± 3c	117 ± 5a	66 ± 2d	1764333 ± 152216c	55 ± 3d	556 ± 40a	67 ± 2d	2982333 ± 313164c
54	25 ± 3c	121 ± 5a	74 ± 3c	1823867 ± 157847b	60 ± 3c	580 ± 41a	76 ± 5c	3131689 ± 328800b
108	31 ± 2a	127 ± 5a	83 ± 3b	1809089 ± 156478b	67 ± 3b	575 ± 41a	86 ± 3b	3146822 ± 312320b
216	27 ± 3b	119 ± 3a	126 ± 4a	1889267 ± 162963a	100 ± 3a	601 ± 43a	127 ± 3a	3244689 ± 340784a

Note: CK: Without stimulation; S: Electrostimulation. Different lowercase letters indicate significant differences (P< 0.05) with the same species using different light intensities (values in the same column)

Table 6: Negative air ion concentration and stomatal quantitative feature of plant species under high-voltage pulsed electrical filed stimulation

Plants	Code	Length (μm)	Width (μm)	Length/width	Area (μm^2)	stomatal density (mm^{-2})	NAI$_C$ (ion·cm^{-3})
Aloe	CK	79.73±4.69b	16.63±3.68a	5.11±1.6b	1041.09±232.51b	22.32±4.89a	63 ± 2b
	S	101.29±5.32a	16.84±3.93a	6.41±1.89a	1338.42±315.67a	21.02±5.09a	1756822± 145112a
Sansevieria	CK	151.32±6.81a	29.10±4.00a	5.31±0.86b	3453.62±467.50a	12.4±2.54a	51 ± 3a
	S	104.98±8.51b	11.54±2.25b	9.48±2.21a	950.92±200.01b	12.98±2.67a	547 ± 4a
Agave	CK	117.00±13.83b	43.30±5.38a	2.74±0.45b	3981.80±715.51b	45.14±4.66a	65 ± 2b
	S	142.60±11.48a	42.50±3.89a	3.38±0.35a	4771.30±668.84a	46.22±4.06a	2952889± 284953a

Note: CK: Without stimulation; S: Electrostimulation. Different lowercase letters meant significant difference (P< 0.05) among the same cultivar in different treatment condition

Stomata morphology of agave under optimal CP stimulation was similar to CK, and the stomata were open in both the case (Fig. 2 E F). Compared to CK, stomatal length under optimal PEF stimulation increased by 22% (P < 0.05). Furthermore, the stomatal width decreased with a small (2%) magnitude reduction (Table 6). The stomatal area significantly increased by 20% (P<0.05, Table 6). No dramatic changes were observed in stomata density after PEF stimulation. The capacity of agave for NAI generation after optimal PEF stimulation was 45,429.1-fold significantly (P < 0.05) higher than that in the CK (Table 6).

Discussion

Under natural conditions, the ability of individual plants to generate NAI is weak in indoors. Given the beneficial biological effects of NAIs, researchers have explored ways to improve the capacity for NAI generation of individual indoor plants. Previous studies have shown that stimulation using high-voltage PEF (Tikhonov *et al.*, 2004; Yang *et al.*, 2011; Zhang *et al.*, 2015) and light intensity significantly improves the ability of plants to generate NAIs (Wang and Li, 2009). With regard to the underlying mechanism, Tikhonov *et al.* (2004) proposed that production of NAIs mainly depended on the physiological processes of the plant but was not closely related to the plant physical form. This is consistent with the results of the present study.

It was found that the capacity of NAIs generation varied with different intensities of PEF. Yang *et al.* (2009) assumed that an optimal PEF was applied to significantly improve NAIs generation of individual plants. Our results demonstrated that there was an optimal CP of PEF for plant NAIs generation. However, each plant species has its own optimal CP of PEF. These findings confirmed the Xi and Yang (2008) assumption, in which the "threshold value" and "power window" of a low-frequency electrical field impart specific biological effects. With a particular stimulation of PEF, the plant grows well, and no damage incurred, while its capacity of NAIs generation significantly improves. However, we noted that for sansevieria, with all tested CPs, its ability to produce NAIs changed slightly. We speculate that this might be caused by the tested CPs, which did not fit well with the inherent potential frequency of this species, because PEF resonance did not occur. Consequently, the effect on NAIs generation of sansevieria was not significant.

Mostly, sufficient voltage stimulation resulted in a burst of NAIs generation. Our findings showed that voltage of the plants were positively correlated with its capacity of generating NAIs. We speculated that voltage accumulated in

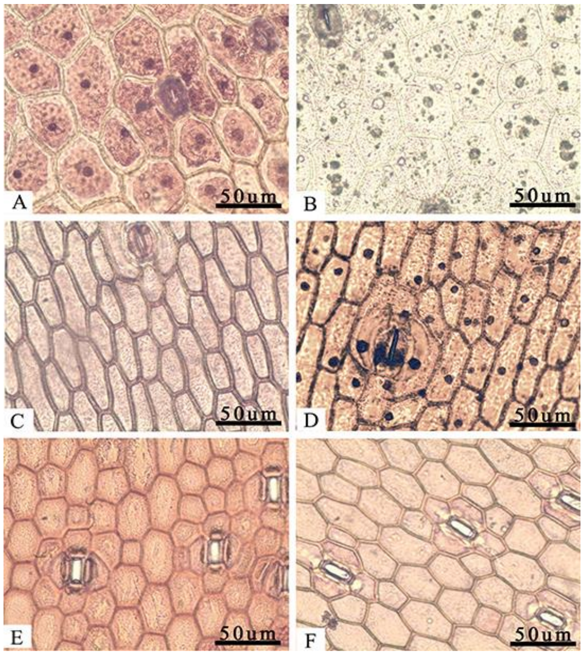

Fig. 2: The stomatal shape feature of three plant species under the stimulation of high voltage pulsed electrical field with optimal combinational parameters (The shape feature of three plant species were observed under 40× magnification. A~B: The stomatal feature of *Aloe vera* var. *chinensis* under normal and electrostimulation conditions, separately; C~D: The stomata feature of *Sansevieria trifasciata* under normal and electrostimulation conditions, separately; E~F: The stomata feature of *Agave americana* var. *variegata* under normal and electrostimulation conditions, separately)

the plant acted as an energy source for opening NAI-generating channels. However, we did not observe a significant variation of NAIC in sansevieria. Again, this probably is due to the high threshold of voltage to induce NAIs producing or unreasonable morphology to generate NAIs. Thus, large scale investigation of NAIs release among different plants should be carried out to uncover a list of appropriate plant varieties for improving indoor NAIC.

NAIs generation in plants is a complex process and is closely related to environmental factors (Tikhonov *et al.*, 2004; Wang and Li, 2009). Various meteorological factors such as illumination, temperature, and humidity may indirectly affect NAIs generation (Mu and Liang, 2009). Our results showed that the illumination level was positively correlated with NAI generation of plants under optimal PEF stimulation. This was consistent with previous study (Wang and Li 2009). Moreover, NAIs release did not significantly differ between 54 μmol·m^{-2}·s^{-1} and 108 μmol·m^{-2}·s^{-1} ($P >$ 0.05), which was a common result among three studied species (Table 6). Further experiments would be conducted to reveal the mechanism of this finding.

Importantly, this study revealed that stomata status of three specie was changed dramatically under optimal PEF stimulation and normal condition. This phenomenon was mostly pronounced in agave, which the NAIs release under optimal PEF was 45,429.1-fold higher than that in the natural condition. Higher stomatal density led to greater NAIs generation capacity. Thus, we believe that leaf stomata are one of the channels involved in NAIs producing by application of PEF stimulation.

Conclusion

NAIs generation by plants is a complex physiological process, which is influenced by a number of factors, including plant voltage, illumination, temperature and barometric pressure. Beyond these, PEF acts as a most efficient artificial intervention for increasing NAIs release. Optimal PEF was required for efficient release of NAIs from plants. Each plant species has its own optimal PEF. The intensity of illumination and plant voltage was positively correlated with NAIs release. Furthermore, the ability of plants to release NAIs is closely related to stomatal opening degree and stoma density on plant leaves.

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References

Goldstein, N.I., R.N. Goldstein and M.N. Merzlyak, 1992. Negative air ions as a source of superoxide. *Intl. J. Biometeorol.*, 36: 118–122

Hirsikko, A., T. Yli-Juuti, T. Nieminen, E. Vartiainen, L. Laakso, T. Hussein and M. Kulmala, 2007. Indoor and outdoor air ions and aerosol particles in the urban atmosphere of Helsinki: characteristics, sources and formation. *Boreal Environ. Res.*, 12: 295–310

Horrak, U., J. Salm and H. Tammet, 2003. Diurnal variation in the concentration of air ions of different mobility classes in a rural area. *J. Geophys. Res.*, 108: AAC7-1–AAC7-11

Iwama, H., 2004. Negative air ions created by water shearing improve erythrocyte deformability and aerobic metabolism. *Indoor Air*, 14: 293–297

Kinne, S.M., 1997. *A Public Health Approach to Evaluating the Significance of Air Ions*. Storming Media, Houston

Kosenko, E.A., Y.G. Kaminsky, I.G.T.V. Stavrovskaya, Sirota and M.N. Kondrashova, 1997. The stimulatory effect of negative air ions and hydrogen peroxide on the activity of superoxide dismutase. *FEBS Lett.*, 410: 309–312

Laakso, L., A. Hirsikko, T. Groenholm, M. Kulmala, A. Luts and T.E. Parts, 2007. Waterfalls as sources of small charged aerosol particles. *Atmos. Chem. Phys.*, 7: 2271–2275

Li, S., S. Lu, B. Chen, Q. Pan, Y. Zhang and X. Yang, 2013. Distribution characteristics and law of negative air ions in typical garden flora areas of Beijing. *J. Food Agric. Environ.*, 11: 1239–1246

Liang, H., X.S. Chen, J.G. Yin and L.J. Da, 2014. The spatial-temporal pattern and influencing factors of negative air ions in urban forests, Shanghai, China. *J. For. Res.*, 25: 847–856

Ling, X., R. Jayaratne and L. Morawska, 2010. Air ion concentrations in various urban outdoor environments. *Atmos. Environ.*, 44: 2186–2193

Mu, D. and Y.H. Liang, 2009. Air negative ion concentration and its relationships with meteorological factors in greenbelts of Jiamusi, Heilongjiang Province. *Chin. J. Appl. Ecol.*, 20: 2038–2041

Shao, H., Q. He, H. Yan, Z. Hou and T. Li, 2005. Spatio-temporal changes of negative air ion concentrations in Beijing. *J. Beijing For. Univ.*, 27: 35–39

Shepherd, S.J., C.B. Beggs, C.F. Smith, K.G. Kerr, C.J. Noakes and P.A. Sleigh, 2010. Effect of negative air ions on the potential for bacterial contamination of plastic medical equipment. *BMC Infec. Dis.*, 10: 92

Singh, K., A. Malik and M. Singh, 2009. Spatial variation in air ion concentrations under different indoor environments. *Intl. J. BioSci. Psychiatr. Technol.*, 1: 36–41

Tikhonov, V.P., V.D. Tsvetkov, E.G. Litvinova, T.V. Sirota and M.N. Kondrashova, 2004. Generation of negative air ions by plants upon pulsed electrical stimulation applied to soil. *Russ. J. Plant Physiol.*, 51: 414–419

Vana, M., M. Ehn, T. Petaja, H. Vuollekoski, P. Aalto, G. de Leeuw, D. Ceburnis, C.D. O'Dowd and M. Kulmala, 2008. Characteristic features of air ions at Mace Head on the west coast of Ireland. *Atmos. Res.*, 90: 278–286

Wang, J. and S.H. Li, 2009. Changes in negative air ions concentration under different light intensities and development of a model to relate light intensity to directional change. *J. Environ. Manage.*, 90: 2746–2754

Wu, C.F., C.H. Lai, H.J. Chu and W.H. Lin, 2011a. Evaluating and Mapping of Spatial Air Ion Quality Patterns in a Residential Garden Using a Geostatistic Method. *Intl. J. Environ. Res. Public Health*, 8: 2304–2319

Wu, R.Y., C.Y. Deng, B. Wang, D.B. Huang, L. Lin, J.M. Huang and J.G. Zheng, 2011b. Study on Indoor Plant Germplasm Resources, which Generate Negative Air Ions. *Chin. Agric. Sci. Bull.*, 27: 91–97

Wu, R.Y., C.Y. Deng, G.L. Xin, H.Y. Weng, Z.J. Yang, T.J.R. Zhu and J.G. Zheng, 2014. Study on the Effect of Indoor Air Quality by Negative Air Ion of Plant. *J. Anhui Agric. Sci.*, 42: 9491–9494

Wu, R.Y., J.G. Zheng, Y.F. Sun, Q.S. Wang, C.Y. Deng and D.P. Ye, 2017. Research on generation of negative air ions by plants and stomatal characteristics under pulsed electrical field stimulation. *Intl. J. Agric. Biol.*, 19: 1235–1245

Xi, G. and Y. Yang, 2008. Non-thermal effect of electromagnetic field on biological system and its mechanism. *Coll. Phys.*, 27: 50–52

Yang, Y., G. Xi, K. Liu and X. Zhang, 2011. Application of Negative High-voltage Pulse Technology to Improve Air Purification Ability of Plants. *High Voltage Eng.*, 37: 190–197

Yang, Y., G. Xi and S. Zhang, 2009. Multiplier effect of pulsed electric field-mediated plant negative air ion launched and its significance. *Coll. Phys.*, 28: 39–42

Zhang, W.C., Z. Shu, J.G. Zheng, R.Y. Wu and C.Y. Deng, 2015. Study on the Negative Air Ions Level Produced by Agave Americana under High-voltage Pulsed Electrical Stimulation. *J. Chongqing Technol. Business Univ. Nat. Sci. Ed.*, 32: 1–5

Zhang, Y., X. Wang and Y. Hong, 2008. Improvement of Slices Technique of Plant Epidermis. *J. Anhui Agric. Sci.*, 36: 12683–12684

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