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Effects of plasma irradiation using various feeding gases on growth of *Raphanus sativus* L.☆

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ABSTRACT

In this work, we have studied the action of dielectric barrier discharge (DBD) plasma irradiation using various feeding gases on seeds of *Raphanus sativus* L. and analysis their growth. Our experimental data shows that Air, O_2, and NO(10%)+N_2 feeding gases plasma irradiation enhanced plant growth, whereas N_2, He and Ar feeding gases plasma irradiation had little influence on plant growth. Moreover, humid air plasma irradiation was more effective in growth enhancement than dry one. More than 2.3 times faster growth was observed by 3 min air plasma irradiation with 40–90% relative humidity. The reactive species generated by plasma in gas phase were detected using optical emission spectroscopy and in liquid phase by electron spin resonance (ESR) spectroscopy. We concluded that OH and O radicals were key species for plant growth enhancement.

1. Introduction

In the last decade, the world agricultural yield increase has not kept pace with the rapid world population growth [1–5]. The demand for food will continue to increase towards 2050 as a result of population growth by an additional 2.7 billion people [6]. Such demand will cause the global food crisis. One possible solution to the global food crisis is to improve agricultural productivity and output. Fig. 1, shows approaches to improve agricultural productivity and output together with potential plasma applications. There are three main categories of improvement methods of the agricultural productivity and output: irrigation, fertilization, and crop protection. Atmospheric pressure nonthermal plasmas can contribute such improvements in the three categories by various ways such as sterilization, fertilization, water treatment and purification, soil treatment, seed treatment, storage improvement, insecticide, pre-harvest treatments, post-harvest treatments, because they provide radicals, ions, electrons, light, as well as electric field without appreciable thermal damage to plants, crops, and fruits [7–17]. Improvement of agricultural productivity has been also tried by gas fumigation, seed treatments using radiations, UV light, laser, electric field, and magnetic field [18–28]. It is important to clarify advantages and disadvantages of plasma treatments over other methods [29–38].

Our group has already reported that dielectric barrier discharge (DBD) plasma irradiation to seeds of radish sprouts (*Raphanus sativus* L.) can induce continuous growth enhancement of the plants after their germination for three weeks [7–9]. We have also shown plasma irradiation to seeds can significantly increase crop yields and shorten harvest time [10]. However, the growth enhancement mechanism has not been clarified yet. Here to obtain information on key species involved into the growth enhancement, we have studied effects of DBD plasma irradiation using various feeding gases to seeds of radish sprouts and analysis their growth. For better understanding we have compared the gas fumigation

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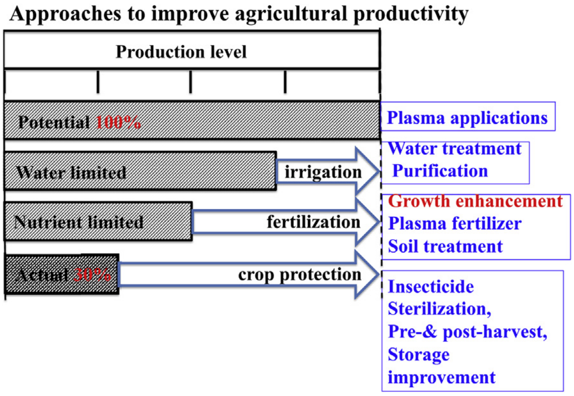

Fig. 1. Approaches to improve agricultural productivity and output together with potential plasma applications.

and plasma irradiation on the growth of radish sprouts. We have also studied the generation of reactive species using optical emission spectroscopy (OES) for various gas feeding plasmas. Additionally, we have also studied the generation of hydroxyl radicals ($^{\bullet}OH$) in DI water after the plasma radiation using various feeding gases using electron spin resonance (ESR) spectroscopy with help of 5,5-dimethyl-pyrroline *N*-oxide (DMPO) spin trap. Latter, we have studied the growth enhancement in humid air plasma. Furthermore, we have also the studied the effect of storage days on plasma irradiation seeds.

2. Material and methods

2.1. Materials

DMPO (5,5-Dimethyl-1-pyrroline *N*-oxide, $C_6H_{11}NO$), Phosphate buffer saline (0.05 M in H_2O, PBS) and Hydrogen peroxide solution (30 wt% in H_2O, H_2O_2) were purchased from Aldrich Chemical Co. (USA). Radish seeds were supplier by Nakamura seeds Co. (Japan).

2.2. Dielectric barrier discharge (DBD) plasma

Experiments were carried out using a scalable DBD device [7–9]. The device was set in a chamber equipped with a rotary vacuum pump and a gas cylinder as shown in Fig. 2(a),(b). The gas in the chamber was air, O_2, NO (10%)+N_2, N_2, He, and Ar at atmospheric pressure. The DBD device consisted of 20 stainless rod electrodes of 1 mm in outer diameter and 60 mm in length covered with a ceramic tube of 2 mm in outer diameter. The electrodes were arranged parallel with a spacing of 0.2 mm. DBD plasmas were generated between the electrodes by supplying a 10 kHz AC high voltage (Logy Electric, LHV-09K). The discharge voltage and current were measured with a high-voltage probe (Tektronix, P6015A) and a Rogowski coil (URD, CTL-28-S90-05Z-1R1), respectively. The peak-to-peak discharge voltage and current were 9.2 kV and 0.2 A. The corresponding discharge power density was 1.49 W/cm^2, which was deduced from a voltage/charge Lissajous plot.

2.3. Treatment condition and experimental setup

For the combinatorial plasma irradiation, dry radish seeds were set in lines parallel with the z axes at $x = -5$, 0, 5, 10, 20, and 30 mm, with 10 seeds per line, as shown in Fig. 2(a). The irradiation was carried out for 3 min at $y = 3$ mm below the electrodes. For comparison, 10 dry radish seeds were fumigated in O_2, NO (10%)+N_2, N_2, He, and Ar for 5, 10 and 20 min. After plasma irradiation and fumigation, seeds were put in a tray filled with deionized water and cultivated for 24 days under dark condition in a plant incubator. The temperature and relative humidity of the incubator were 22 °C and 80%rh, respectively. The total length of radish sprouts from primary root to coleoptile was measured. The dry weight were measured after 7 days cultivation and set in vacuum for 5 days. The whole experiments were carried out three times to confirm reproducibility. Effects of humid air plasma irradiation were examined using plasma irradiation in an incubator with 10–90%rh. Cultivation conditions were the same as the ambient gas experiments.

2.4. OES and ESR analysis

To obtain information on species generated in plasmas, optical emission spectra of plasmas were measured with an optical multichannel analyzer (Ocean Optics, USB2000+) equipped with a quartz optical fiber as shown in Fig. 2(b). The spectra were obtained with a 0.1 s integration time and the cumulative number of measurement performing times was 10.

OH radicals generated in water by plasma irradiation were detected with an electron spin resonance meter (Bruker, ER041X X-band). DMPO (5,5-Dimethyl-1-pyrroline *N*-oxide, $C_6H_{11}NO$) was used as a radical trapping agent [40–45]. The concentration of DMPO is 0.1 M in distilled water (DI water). The distance between DBD discharge plasmas and DI water was 3 mm. After 10 min plasma irradiation, 800 μl of the solution was employed for ESR measurements. ESR measurements were carried out at a 9.806322 GHz microwave frequency, a 335.54 s sweep time, and 100 kHz modulation frequency. The conditions for measuring DMPO included a magnetic field of 348.0 mT, a modulation width of 0.1 mT, and a time constant of 0.168 s. The cumulative number of measurement performing times was 200.

3. Results and discussion

3.1. Effects of various feeding gases plasma on growth of radish sprouts

To obtain information on key species involved into the growth enhancement, we have examined effects of plasma irradiation to seeds in various feeding gases such as air, O_2, NO(10%)+N_2, N_2, He, and Ar. Fig. 3(a)–(f), show growth curves of seedling for 24 days cultivation after plasma irradiation together with gas fumigation and control. Table 1 summarizes normalized seedling length of radish sprouts, namely the length ratio to control, after 7 days cultivation for gas fumigation and plasma irradiation using air, O_2, NO (10%)+N_2, N_2, He, and Ar gases. For gas fumigation, O_2 gas fumigation of 5 and 10 min shows around 10% growth enhancement, whereas fumigation of all other conditions has little effect. O_2 concentration is one of the main factors affecting respiration of seeds and hence O_2 gas fumigation slightly enhances plant growth.

Among all treatment, air plasma irradiation is the most effective to growth enhancement. The normalized length for air, O_2 and NO(10%)+N_2 plasma irradiation are 1.42, 1.24 and 1.28 (Tukey test, $N = 10$, $P < 0.1$), respectively. This shows that among all gases plasma treatment, the air gas plasma treatment was most effective for growth enhancement. However, the average length for seedling is longer than that control for O_2 gas under fumigation treatment for 10 min. Whereas, the average of sprout seedlings that been irradiated by others gas are nearly as that the control. For plasma irradiation using Air, O_2 and NO (10%)+N_2 feeding gases help in promote growth of plant, whereas N_2, He and Ar feeding gases plasma irradiation doesn't have any effect on growth of plant. The

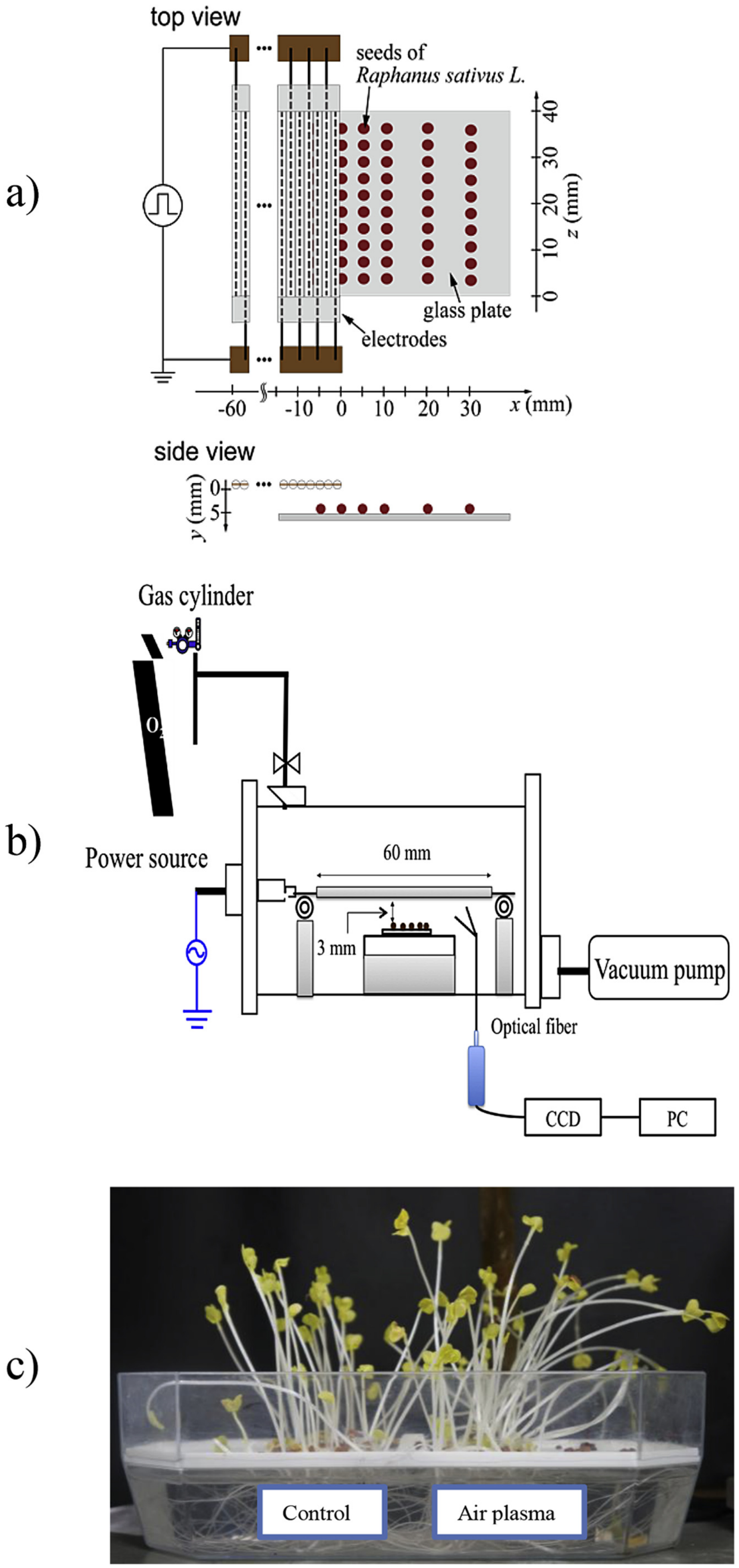

Fig. 2. Schematic diagram of (a) scalable atmospheric pressure DBD device, (b) experimental setup with a chamber and (c) Photograph of Radish sprouts after 7 days cultivation by air plasma irradiation.

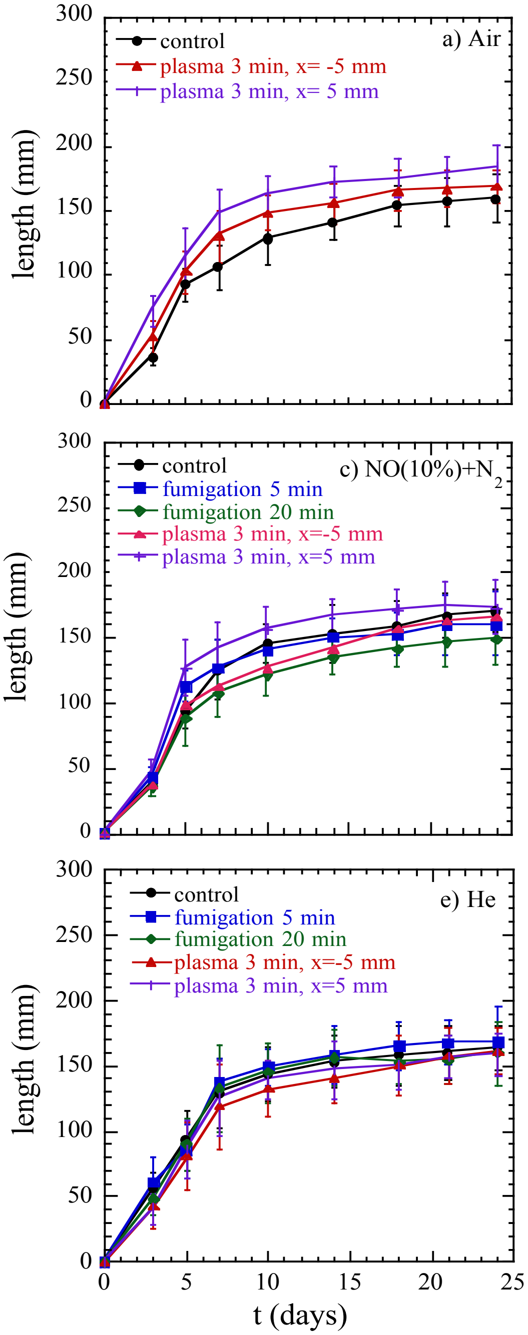

Fig. 3. Growth curves of average seedling length for plasma irradiation in dry air (a), O_2 (b), NO (10%)+N_2 (c), N_2 (d), He (e), and Ar (f) for 24 days cultivation. $N = 10$, $p < 0.1$.

Table 1
Normalized length of seedling of radish sprouts after 7 days cultivation for gas fumigation and 3 min plasma irradiation in dry air, O_2, NO (10%)+N_2, N_2, He, and Ar. $N = 10$, $P < 0.1$.

Ambient gas species	Gas fumigation time (min)			Position x of plasma irradiation (mm) $t_{on} = 3$ min				
	5	10	20	−5	0	5	10	20
Air				1.18	1.22	1.42	1.28	1.12
O_2	1.09	1.12	1.02	0.87	0.95	1.24	1.36	1.12
NO (10%)+N_2	1.05	0.92	0.86	0.91	0.93	1.28	1.16	1.04
N_2	0.97	0.92	1.02	0.98	0.90	0.96	1.05	0.96
He	1.04	0.94	1.06	0.89	1.01	0.86	1.10	1.02
Ar	0.97	1.07	1.02	1.01	1.04	0.98	0.97	1.08

experimental results were analyzed by using Tukey test to evaluated that are significant difference from others gases. The difference increases with time after 3 days. The ratio increases to 1.42, 1.24 and 1.28 for air, O_2 and NO (10%)+N_2 plasma irradiation after 7 days (Tukey test, $P < 0.1$), as shown in Table 1. Table 1 shows comparison between fumigation and plasma irradiation treatment on plant growth using various feeding gases. Plant growth rate was nearly decreases after 24 days. The radio decreases to 1.15, 1.09 and 1.04 for air, O_2 and NO (10%)+N_2 gases plasma irradiation, respectively. Fig. 4, shows the average weight of sprouts after 7 days cultivation. The maximum average weight of sprouts is 1.22 g for O_2 plasma irradiation condition. The increase in weight of irradiated seeds is about 10% for O_2, Air and NO (10%)+N_2 gases plasma

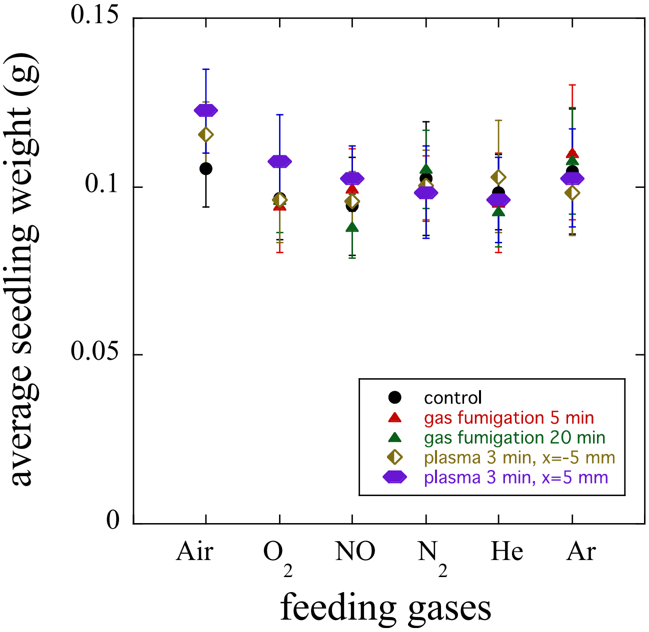

Fig. 4. Average weight of radish sprouts after 7 days cultivation by various gases fumigations and various feeding gases plasma irradiation species. N = 10.

irradiation than without irradiation seeds. While, N_2, He and Ar gases plasma irradiation have similar action to without plasma irradiation.

3.2. *Optical emission spectra of plasma*

We measured optical emission spectra of plasmas, because they provide two kinds of information: light emitted from plasmas and electron impact generation of radicals and excited species including metastable species. Typical optical emission spectra of dry air, O_2, NO(10%)+N_2, N_2, He, and Ar gases plasmas are shown in Fig. 5. One important common feature in these spectra is the fact that there are no emission spectra from impurities. Especially, no OH emissions exist in all spectra, indicating residual H_2O concentration in the chamber is negligible. In other words, all these discharge plasmas are dry ones. All these emission spectra also show generation of excited atoms and suggest generation of high energy electrons, ions, and metastable species in the plasmas.

The emission spectrum of dry air plasma in Fig. 5a, contains strong second N_2 positive system ($C^3\Pi_u \rightarrow B^3\Pi_g$) as well as weak N_2 first negative system, weak N_2 first positive system ($B^3\Pi_g \rightarrow A^3\Sigma_u^+$), and weak NOγ emission. $A^3\Sigma_u^+$, $B^3\Pi_g$, and $C^3\Pi_u$ electronic states exist at 5.5, 7.4 and 11.0 eV above the ground electronic state, respectively [46]. Therefore, N_2 emissions indicate that the dry air plasma contains moderate density of electrons above 7.4 eV and low density of electrons above 11.0 eV. Since onset energy of electron impact dissociation of N_2 is 9.75 eV [47], some N_2 are dissociated into N. However, N emission lines are not detectable in the spectrum, suggesting dissociation degree of N_2 is very low. The emission spectrum of O_2 plasma in Fig. 5b, shows atomic O lines, suggesting some O_2 are dissociated into O due to electron impact dissociation. Since O_2 plasma irradiation brings about growth enhancement [8], O atom is one strong candidate key species to growth enhancement. The intensity in Fig. 5b, is enlarged by 100 and therefore photon flux is very low compared with other plasmas in Fig. 5. In addition, O_2 plasma provides little flux of UV photons.

The emission spectrum of NO(10%)+N_2 plasma in Fig. 5c, contains strong second N_2 positive system as well as weak N_2 first negative system and weak NOγ emission. N lines are not detectable in the spectrum, suggesting dissociation degree of N_2 is very low. The emission spectrum of N_2 plasma in Fig. 5d, contains strong second N_2 positive system as well as weak N_2 first negative system and weak N_2 first positive system. N lines are not detectable in the spectrum, suggesting dissociation degree of N_2 is very low. Although emission spectra of air, NO(10%)+N_2, and N_2 plasmas are very similar spectral profiles and intensities; air and NO(10%)+N_2, plasma irradiation promote plant growth, whereas N_2 plasma irradiation does not. These results clearly show that N_2 emissions and excited species doesn't play any important roles in growth enhancement.

The emission spectrum of He plasma in Fig. 5e, contains He atomic lines and that of Ar plasma in Fig. 5f, contains Ar atomic lines, respectively. In these spectra of rare gas plasmas, no excimer emissions and weak emission lines exist in 200–500 nm range, whereas strong emission lines exist in 500–900 nm range. These results show very low flux of high energy photons provided by these rare gas plasmas. As described in the Section 3.1 irradiation of He plasma and Ar plasma has little effects on plant growth. Therefore, for He and Ar gases plasma the excited state of He (He metastable state) and Ar excited state (Ar metastable state) has no significant contribution for growth enhancement of plant.

3.3. *Effects of irradiation of light from plasma*

As shown in 3.2, plasma provides light in a wide spectra range. Among the wavelength ranges, UV light has important direct effects on biological photochemical reactions due to the high photon energy [39]. Moreover UV light has indirect effects, namely, UV light generates radicals in gas phase or in liquids and then such radicals react with living organisms. To understand that, we studied direct and indirect effects of UV light irradiation on growth enhancement.

Since most biological responses induced by UV light irradiation are highly wavelength-dependent, UV light is categorized into three wavelength regions: UV-A (400–315 nm), UV-B (315–280 nm), UV-C (<280 nm). Among the spectra shown in Fig. 5(a)–(f), air plasma provides the highest intensity in UV-B and UV-C regions. Therefore, we examined effects of irradiation of light from air to seeds on plasma growth enhancement by setting a quartz glass plate between the plasma and seeds. The results showed that light irradiation does not play an important role to growth enhancement (data not shown). This is consistent with our previous experiments using low pressure O_2 rf discharge plasmas [9].

We also evaluated indirect contribution (contribution to radical generation) of light irradiation to growth enhancement. For this purpose, we used a quartz glass plate between air plasma and distilled water (DI water). After the irradiated light from plasmas, we have added the DMPO (spin trapping agent) to DI water. The ESR spectra of DMPO (0.1 M) in DI water solution together with control for the above treated conditions are shown in Fig. 6. Under this condition we have not observed any clean signal of DMPO-OH spin adduct for 10 min, 20 min and 30 min for plasma UV irradiation treatment, as shown in Fig. 6. However, for air plasma treatment, we observed good DMPO-OH spin adduct signal as described in latter section. Therefore, UV light irradiation from plasma has no significant effect on growth enhancement.

3.4. *Detection of OH radical by ESR spectra in DI water after plasma treatment using various feeding gases*

We irradiated plasma to DI water to obtain ESR spectra of

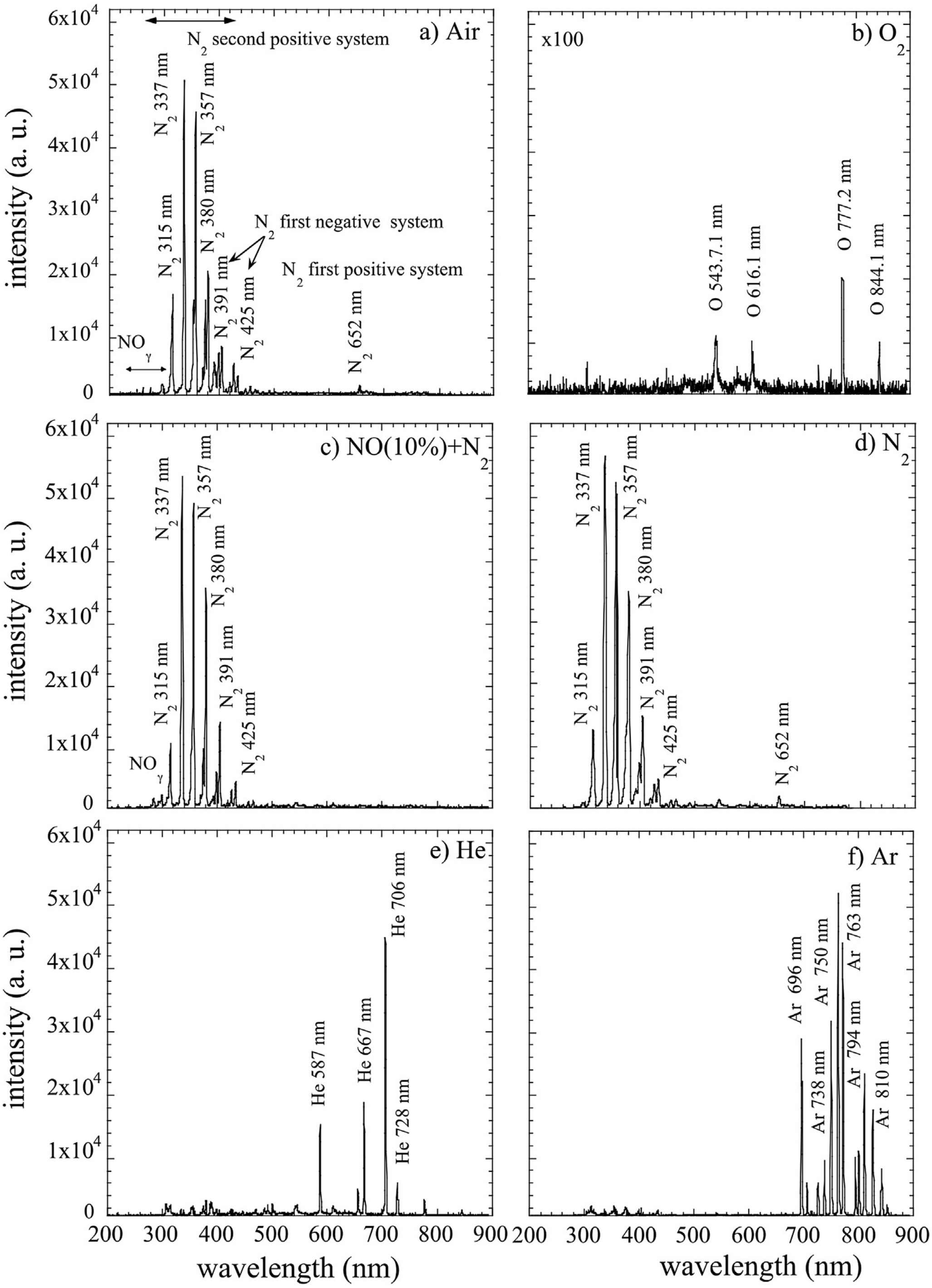

Fig. 5. Plasma spectra obtained in dry air (a), O_2 (b), NO(10%)+N_2 (c), N_2 (d), He (e), and Ar (f).

radicals using DMPO. For this purpose, first we irradiated plasma to DI water for 10 min using Air, O_2, NO(10%)+N_2, N_2, He, and Ar feeding gases. Just after the irradiation we added DMPO (0.1 M) to the plasma treated DI water. The results are shown in Fig. 7(a)–(f). ESR signal of DMPO spin adducts DMPO-OH is denoted as *. ESR spectrum of DMPO-OH spin adduct is clearly detected for all ambient gas. It should be noted that DMPO can trap other radicals such as OOH, O_2^-, no such signal is detected, indicating the concentrations of other radicals is very low. These results show selective generation of OH in DI water by these plasma irradiation. DMPO-OH intensities are highest for O_2 gas plasma as compared to feeding gases plasma. Contribution of electrons and ions to radical generation in liquids is negligible, because remote plasma configuration was employed in this study. Therefore, metastable species in Ar, He, N_2 and NO(10%)+N_2 gases plasmas generate •OH in DI water. Moreover, generation of OH radicals for Air and O_2 gases plasma by various reactions, were described in our early work [43].

Fig. 8, shows the ESR signal intensity of DMPO-OH as a function of air plasma irradiation time. The intensity increases with the

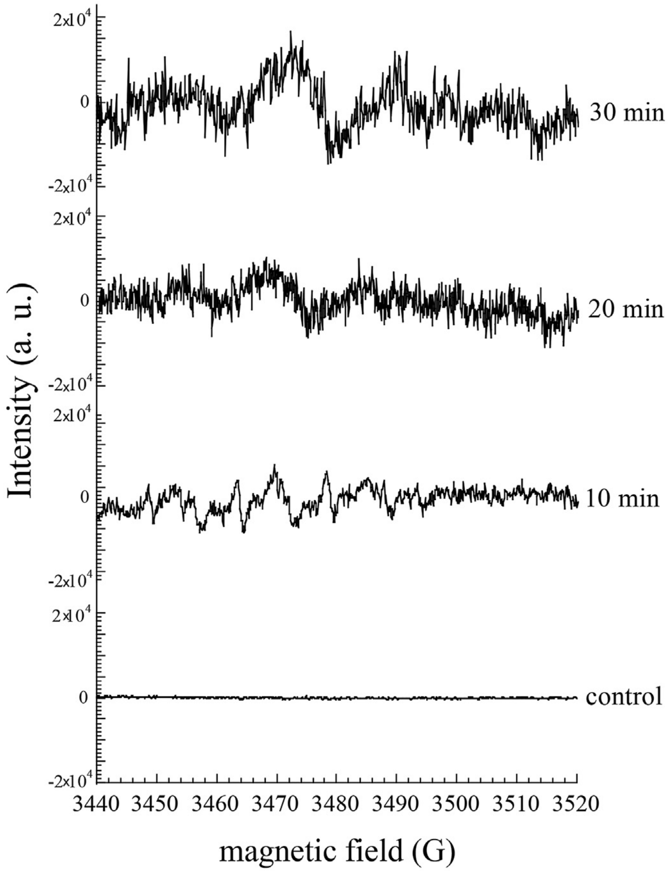

Fig. 6. ESR spectra of DMPO in DI water solution for 10, 20, 30 min UV light irradiation using air DBD plasma together with control (no irradiation).

irradiation time up to 20 min, and then they tend to be saturated for the irradiation time from 20 to 30 min. The latter saturation phenomena are attributed to the temperature rise of the ceramic tubes surrounding the electrodes, because such temperature rise has influence on chemical reactions around the tubes. However, in present study we studied the plasma irradiation time below 10 min, and hence the dose of the radicals is nearly proportional to the irradiation time. In our previous work, we showed the growth enhancement ratio increases as the air plasma irradiation time increases from 0 to 3 min, and it decreases with increasing further the irradiation time [10]. These results clearly indicate that appropriate dose was needed to stimulate growth.

3.5. *Effects of humid air gas plasma irradiation on growth of radish sprouts*

As we described early, in Section 3.1, air plasma irradiation was the most effective to growth enhancement. Ambient air normally contains water vapor, so we have studied effect of humid air gas plasma irradiation to seeds for growth enhancement. Humidity dependence of seedling length normalized by that of control plants is shown in Fig. 9. The normalized length increases form 1.2 for 10% rh to 2.4 for 40%rh and it decreases slightly to 2.3 for 90%rh. Thus humidity was an external key parameter to control growth enhancement. The growth enhancement depends on humidity in 10–40%rh, whereas it keeps the similar growth enhancement level in 40–90% humidity. Therefore, plasma treatment to realize growth enhancement is obtained in the wide humidity range of 40–90%rh. Additionally, these results depicted in Fig. 8 that reactive species originated from H_2O molecules play an important role in growth enhancement.

3.6. *•OH emission spectra of humid air plasma*

The generation of OH radicals in humid air plasma was possible by different reactions, some of major reactions are given below [43,47,48].

$$H_2O + e \rightarrow OH + H + e \tag{1}$$

$$H_2O + O(^1D) \rightarrow 2OH \tag{2}$$

$$N2\ (A^3\textstyle\sum_u^+) + H_2O \rightarrow OH + H + N_2 \tag{3}$$

$$H + O_2 \rightarrow OH + O \tag{4}$$

$$O_3{}^{\bullet -} + H^+ \rightarrow O_2 + OH \tag{5}$$

where e represents an electron and $O(^1D)$ is an O atom in an excited singlet state, generated by the electron impact dissociation of O_2. Emission of O atoms cannot be detected in spectra of humid air plasma (not shown here). Moreover, $O(^1D)$ is rapidly de-excited to $O(^3P)$ in a ground state, by collision with N_2 or O_2. Therefore the process (2) is minor in the plasmas. The spectra in 290–320 nm regions for 10–90%rh are shown in Fig. 10(a). There is the OH $A^2\Sigma^+$-$X^2\Pi$ emission in 305–310 nm region [49], indicating generation of OH in humid air plasmas.

•OH emission intensity dependence of normalized seedling length at 3 days cultivation, as displayed in Fig. 10(b). •OH emission intensity is proportional to generation rate of OH radicals in plasmas. After the generation, some OH radicals are transported to seeds and others react with other atoms and molecules in gas phase. Among gas phase reactions of OH radicals, the following reactions are predominant when the OH radical density is high,

$$OH + OH + M \rightarrow H_2O_2 + M, \tag{6}$$

$$OH + OH \rightarrow H_2O + O, \tag{7}$$

where M represents the third body [48]. The OH radical density tends to be saturated due to these self-recombination reactions, when OH generation rate is high. Since these reactions become less frequent with decreasing the OH radical density, OH radicals in a low density still exist even far from the OH generation region.

3.7. *ESR spectra of DI water irradiated by humid air plasma*

We measured ESR spectra of DMPO (0.1 M) in DI water solution for 10 min humid air plasma irradiation. ESR signal of DMPO spin adducts DMPO-OH is clearly detected, whereas DMPO spin adducts of other radicals such as OOH, O_2^- cannot be detected (not shown here), indicating the concentrations of other radicals is very low. Fig. 11(a), shows humidity dependence of DMPO-OH intensity. The intensity increases with increasing humidity from 10% to 50% and then they decrease slightly with further increasing humidity from 50% to 90%.

Fig. 11(b), reveals the DMPO-OH intensity dependence with normalized seedling length at 3 days cultivation. The normalized length increases from 1.15 to 2.4, being proportional to the DMPO-OH intensity and then the normalized length decreases slightly from 2.4 to 2.1. The normalized length of 2.4 may be the maximum obtained by plasma irradiation. OH radicals were probably one of key reactive species to growth enhancement. Recombination of •OH generates stable H_2O_2, as shown in Equations (6) and (7). Therefore,

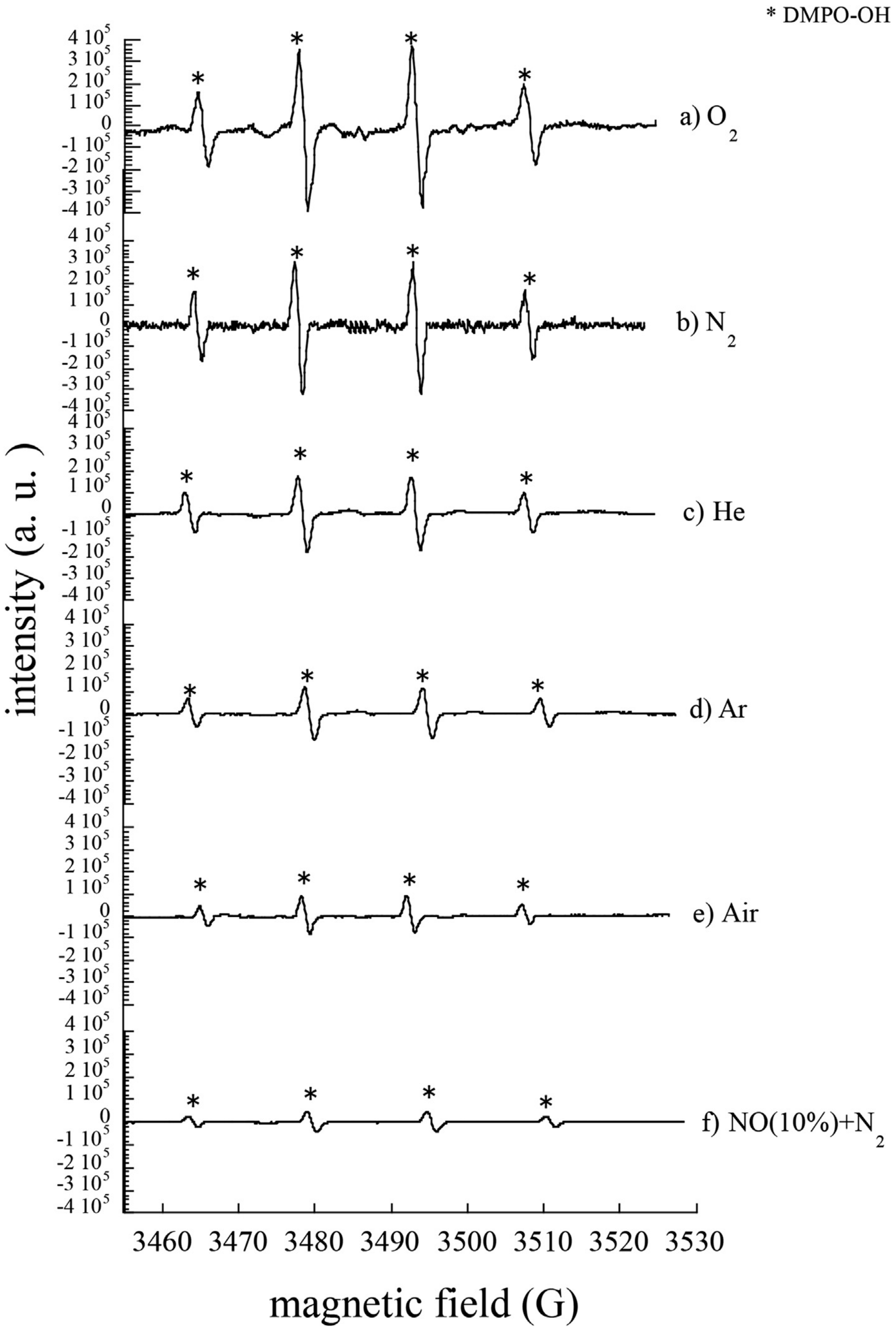

Fig. 7. ESR spectra of DMPO in DI water solution for 10 min plasma irradiation in O_2 (a), N_2 (b), He (c), Ar (d), Air (e), and NO (10%)+N_2 (f). ESR signal of DMPO spin adducts DMPO-OH is denoted as *.

we expected H_2O_2 concentration in DI water increases as DMPO-OH intensity increases. Such expectation is obtained in Fig. 12(a). Latter, we examined correlation between the normalized length and H_2O_2 concentration as shown in Fig. 12(b). Fig. 12b shows that there was no correlation between H_2O_2 and growth factor, hence we can concluded that H_2O_2 was not key factor for the growth enhancement.

3.8. Key species of growth enhancement in humid air plasma

Based on the experimental results in the previous sections, main reactive species formed in humid air plasmas is drawn schematically in Fig. 13 [42]. Main gases in humid air are N_2, O_2, and H_2O. In plasmas, they are dissociated into N, O, OH, and H though electron impact collisions. After chemical reactions, more complex reactive species are formed. Main reactive species formed in humid air plasmas become O, O_3, OH, H_2O_2, NO, NO_2, HNO_2, and HNO_3.

As shown in Table 1 and Fig. 3(b), pure O_2 plasma irradiation to seeds enhances growth. Candidate key species to this enhancement are O_3 and O. Our previous experiments using low pressure rf plasmas suggested that O helps in seeds enhancement [9]. We have already reported in our early work about the correlation between growth enhancement and reactive species such as O_3, NO, and NO_2

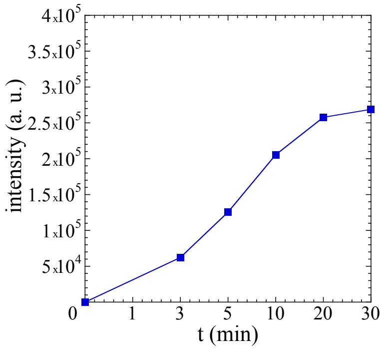

Fig. 8. Dry air plasma irradiation time dependence of radical concentration of DMPO-OH. y = 3 mm.

[10]. Therefore, we concluded O was also one of key species to growth enhancement. As shown in Section 3.5, irradiation of air plasma with an appropriate humidity to seeds increases significantly plant growth, therefore •OH and H_2O_2 are additional candidate key species to growth enhancement. OH correlates strongly with growth enhancement as shown in Figs. 10(b) and 11(b). Although H_2O_2 density increases with OH density as shown in Fig. 12(a) but H_2O_2 doesn't correlated with the normalized length as shown in Fig. 12(b).

Therefore, we identify that O and •OH are key reactive species to growth enhancement. In Table 2, we summarized the electrochemical potential of some reactive species in humid air plasma [9]. However, Table 2 and experimental data reveals that •OH was major key reactive species to growth enhancement because of OH radicals are generated in higher density for humid air plasma and also OH radical has highest high electrochemical potential among other reactive species know till now.

3.9. Effects of storage day of seeds after plasma irradiation on growth enhancement

Finally, we have studied effects of storage day of seeds on growth enhancement, because chemical changes in seeds can take place during storage above 10 days [50] and such changes may have influence on growth enhancement ability of seeds with plasma irradiation. The seeds with and without air plasma irradiation were stored at room temperature and then they were cultivated. The growth enhancement in this section is defined as the ratio of seedling length with plasma irradiation to that without plasma irradiation using seeds of the same storage days. The growth enhancement of storage seeds is normalized by that of immediate cultivation. Fig. 14, shows the storage duration dependence of normalized growth enhancement at 7 days cultivation. Even for long storage duration of 17 months, seeds with air plasma irradiation still keep growth enhancement ability. These results clearly indicate that although short lifetime radicals generated in plasma induces the growth enhancement and induced modifications have long lifetime. We need further study to clarify the long lifetime modifications induced by plasma irradiation.

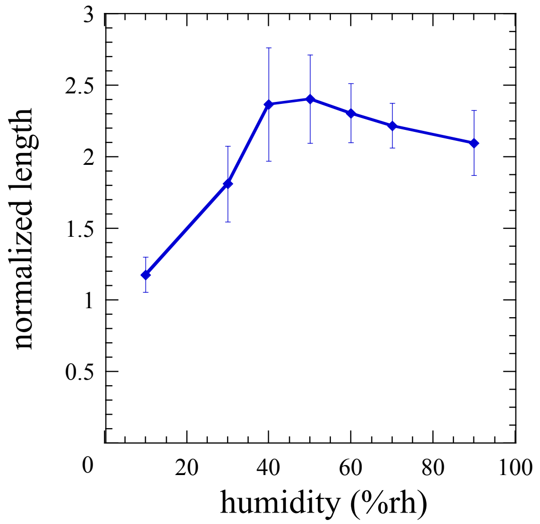

Fig. 9. Normalized length of radish sprout on humidity dependence by air plasma at 3 days cultivation. N = 10, p < 0.1.

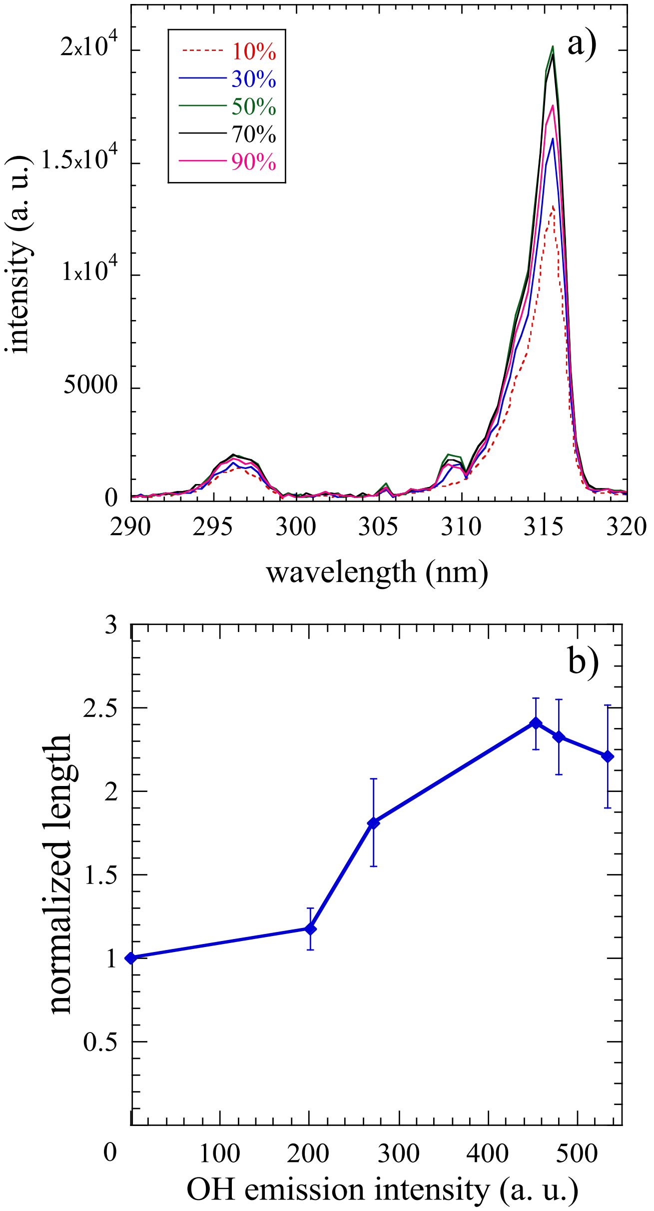

Fig. 10. Spectra containing OH emission in 305–310 nm region in air plasmas with 10–90%rh (a) and dependence of normalized seedling length at 3 days cultivation on OH emission intensity in 305–310 nm. N = 10, p < 0.1(b).

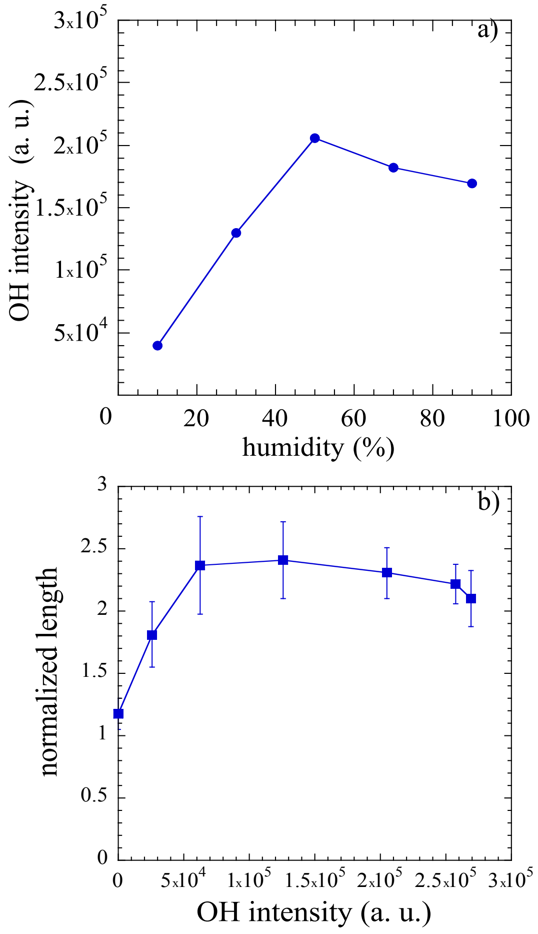

Fig. 11. Humidity dependence of DMPO-OH intensity for 10 min air plasma irradiation. x = −5 mm, y = 3 mm (a), OH intensity dependence of normalized seedling length at 3 days cultivation. N = 10, p < 0.1. (b).

4. Conclusion

This study demonstrated the importance of various gases and humidity on plasma induced growth enhancement. The following conclusions are obtained in this study.

1. Air, O_2, and NO (10%)+N_2 gases plasma irradiation enhance their growth, whereas N_2, He and Ar gases plasma irradiation do not have significant effect on growth enhancement.
2. O_2 gas fumigation enhances their growth, while NO(10%)+N_2, N_2, He, and Ar gases fumigation do not.
3. 3 min irradiation of air gas plasma with 40–90%rh leads to more than 2.3 times faster growth.
4. OH and O are key species to growth enhancement based on growth enhancement.
5. Radicals in gas were detected by optical emission spectra, while OH radicals detected in solution using ESR
6. Even for the long storage duration of 17 months, seeds with air plasma irradiation still keep growth enhancement ability.

Hence, plasma irradiation has strong influence on growth enhancement and it can be improved by changing the feeding gases and humid conditions Therefore, plasma can play important role in agriculture development.

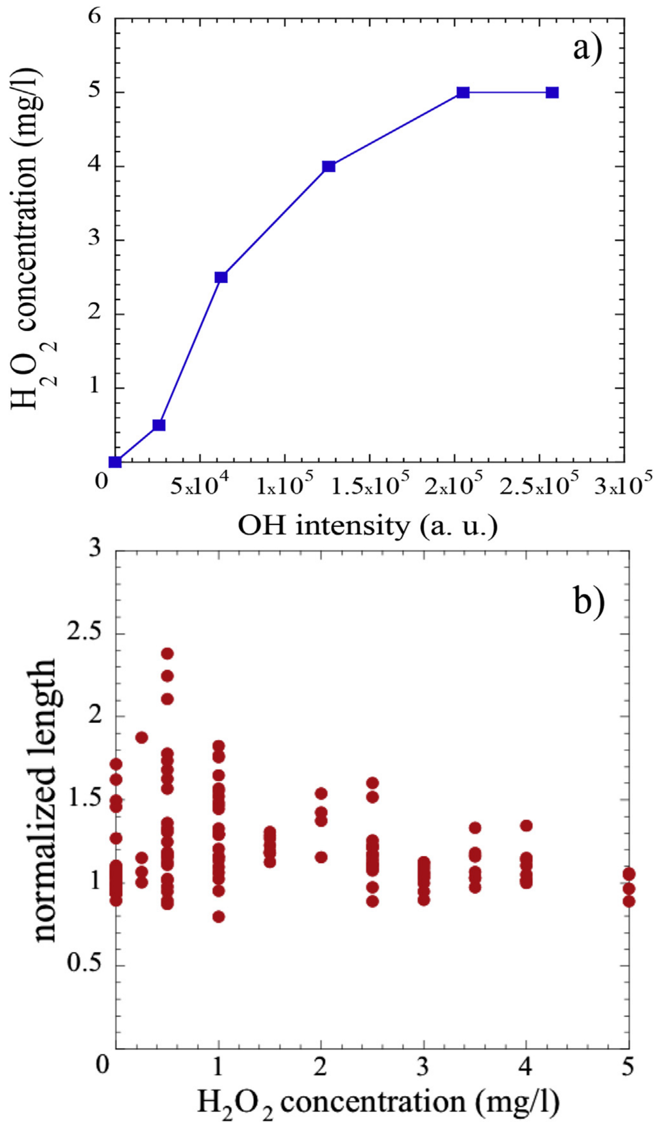

Fig. 12. H_2O_2 concentration dependence on OH intensity (a) and H_2O_2 concentration dependence of normalized seedling length at 3 days cultivation (b). N = 10, p < 0.1.

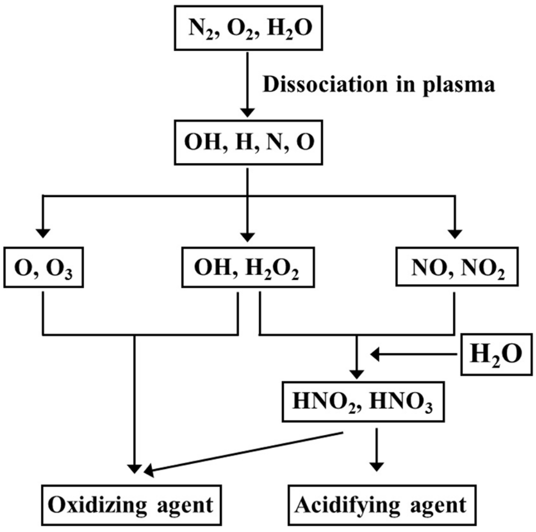

Fig. 13. Schematic diagram of main reactive species formed in humid air plasma.

Table 2
Electrochemical potential values of important reactive species generated in humid air plasma along with their concentration, 3.3×10^{16} cm^{-3} correspond to 1 ppm.

Species	Chemical formula	Electrochemical potential (V)	Concentration (cm^{-3})
Hydroxyl radical	OH	+2.59	10^{15}–10^{17}
Ozone	O_3	+2.07	10^{16}–10^{17}
Hydrogen peroxide	H_2O_2	+1.77	10^{14}–10^{16}
Perhydroxyl radical	HO_2	+1.49	Not detected
Oxygen molecule	O_2	+1.23	10^{22}
Hydroperoxide anion	HO_2^-	+0.88	Not measured
Superoxide anion	O_2^-	−0.33	Not detected

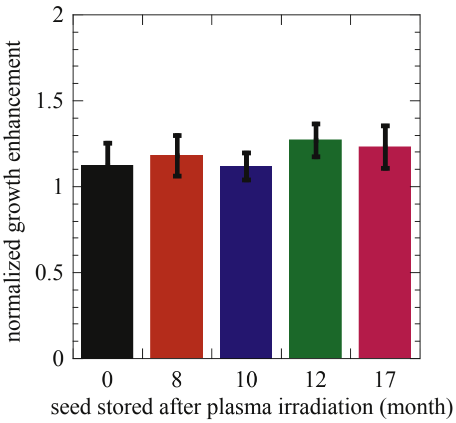

Fig. 14. Storage duration dependence to normalized growth enhancement after 7 days cultivation. N = 50.

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