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Response of lettuce seeds undergoing dormancy break and early senescence to plasma irradiation

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This study reports the response of lettuce seeds undergoing dormancy breaking and early senescence to DBD plasma irradiation. A heat map of germination percentages at 12 hours revealed that the dormancy breaks by 39 days of storage and a 1-min plasma irradiation enhances germination for dormant seeds. Plasma irradiation does not affect already dormancy-broken seeds. Early senescence by storage was estimated by ESR measurements and molecular modification of quercetin. This study revealed that lettuce seed susceptibility to plasma irradiation depends on storage time and conditions, with dormancy state as a critical variable modulating the impact of plasma irradiation.

Plasma irradiation has gained attention as an innovative method for improving agricultural productivity[1),2)3)]. The irradiation of plant seeds with plasma can promote growth and increase harvest yield[4)]. We have demonstrated that three minutes of plasma irradiation (2.3 W in discharge power) of *Arabidopsis thaliana* seeds results in growth enhancement at all growth stages from their germination to harvest[5)]. Growth response induced by seed treatment with plasma has also been shown in numerous other plants, for example, radish[6)7)], wheat[8)], red clover[9)], sunflower[10)], red clover, and purple coneflower[11)]. Furthermore, studies on the molecular mechanisms underlying such effects (reviewed recently by Mildaziene et al. [12)]) have revealed that plasma irradiation alters the balance of abscisic acid (ABA) and gibberellic acid (GA) in seeds [13)14)] and deoxyribonucleic acid (DNA) methylation levels in seeds[15)]. Recently, a method for directly detecting the reactive oxygen and nitrogen species (RONS) induced in seeds by plasma irradiation using mass spectrometry has been created to quantitatively estimate the biological effects of plasma irradiation[16)]. Most of the research uses plasma to induce plant response. Research on plasma agriculture, however, has focused on the correlation between phenotype of plants and plasma conditions such as voltage, power and gas configuration. It has been pointed out that understanding the mechanism of the plasma-induced plant response is crucial and is based on the knowledge of plant molecular biology. Research regarding dormancy from the viewpoints of RONSs has been carried out since the dormancy state of seeds is one of the most essential physiological mechanisms controlling the germination[17)]. Bailly et al. showed that seeds produce endogenous ROS through the time of storage after seed harvesting[18)] and the storage conditions (such as humidity[19)]) can alter their dormancy state. However, there have been limited reports discussing plant response induced by seed irradiation with plasma and regarding dormancy as a relevant parameter. In this study, we provided new insights into the effects of plasma irradiation, focusing on their dependence on changes in the seed dormancy state.

Seeds of *Lactuca sativa* L. (lettuce) were purchased from Asahi Farm, Japan. Lettuce seeds were selected as a model of seeds whose dormancy state changes in a short period of time[19)]. The seeds of the same lot delivered on the same day were used for all experiments of this study. Physiological state of seeds was modulated by two methods: humidification and storage time. Humidification was carried out by leaving the seeds in the dark for 0, 0.5, 1, and 2 days at a temperature of 22°C and a humidity of 85 %Rh [19)] to enable a short-term study on the effects of plasma irradiation. For the estimation of storage effects, seeds were preserved in the dark at 4 °C in sealed bags containing 20 mL of seeds for 152 days. During the storage period, one of the bags was used for each germination test. An experiment for the stored-seeds was conducted to study the relationship between the plasma irradiation and germination without the humidification. A scalable dielectric barrier discharge (SDBD) electrode was used for irradiating seed with plasma [4),16),20),21)]. Lettuce seeds (3000 mg or about 3500 seeds) were distributed equally on a quartz plate placed under the SDBD electrode so that the distance between the electrode and the seed surface was 5 mm. Temperature and humidity were maintained at 23.6 ± 2.3 °C and 52.9 ± 6.1 %Rh, respectively. Plasma has been generated intermittently (5 s ON / 55 s OFF) near the electrode using 13 kV_{pp} in the discharge voltage with 9.4 kHz in the reputation frequency. The discharge power was measured by V-Q Lissajous method using a capacitor inserted between the SDBD electrode and the ground. The ozone (O_3) concentration was measured with the same manner as in reference [22)] . The O_3 concentration was measured using O_3 detection tubes No. 18 M (Gastec, Kanagawa, Japan) connected to a hole of glass plate vertically placed at 5 mm below from the center of the SDBD electrode through a silicon rubber tube. The indicated O_3 concentrations were corrected by the dead volume of the rubber tube to the hole. O_3 concentration was separately obtained at 0, 10, 30, and 50 s elapsed after the plasma generation for 5 s. For the germination test, the samples of seeds (450 mg or about 520 seeds) were taken out from the plate after 1, 3, and 5 minutes of irradiation. For the test, 30 seeds were placed in a 5 x 6 array with tweezers on a filter paper (Advantec; 00021090) soaked with 3 ml of tap water and put on a Petri dish (Kanto Kagaku; CSPD90-15S). The germination test was carried out in an incubation chamber under controlled conditions: temperature of 22°C, a humidity of 55%, and a photon flux of 100 $\mu mol/m^2s$. The number of germinated seeds was counted every 12 h. Number of seeds for one replicate was 50, and 3 biological replicates. A water content of seeds was measured with an infrared moisture analyzer (Kett; FD-660) using 1.0 g or about 1160 seeds (the number of experimental replications was 14). An electron spin resonance (ESR) spectroscopy (Bruker, ER072) was carried out to measure an amount of organic radicals in seeds[21),23),24)] using microwave power 2.15 mW, frequency 100 kHz, g-factor range from 1.84 to 2.19, gain 1.00 x 10^5, temperature 300 K, and data points 1024. For the ESR measurements, seed samples employed for the germination test were used. The ESR signal was divided by the weight of 50 seeds (see Table S2). Means of various parameters between the control and treatment groups were compared using Student's *t*-tests for independent samples.

The characteristics of SDBD plasma was evaluated in terms of power consumption and O_3 concentration. The discharge power of SDBD plasma was 15.8 ± 0.96 W. Fig. 1 shows O_3 concentration. In Fig. 1, $t = 0$ stands for the time immediately after plasma generation for

5 s. The O_3 concentration was 97.0 ppm at 0 s and gradually decreased afterword. The O_3 concentration was below the limit of detection at 4 ppm at 50 s. The maximum O_3 concentration remained at 93.5–97.0 ppm when 5 s of plasma generation was performed 12, 36, and 60 times with an OFF-time interval of 55 s. The seeds thus experienced multiple pulses of O_3 exposure that decayed within approximately 50–55 s. The O_3 exposure amount was evaluated by integrating the result of Fig. 1, assuming that the slope from 30 s to 50 s is maintained until 60 s. As a result, when one cycle of 5 s ON / 55 s OFF is performed, the O_3 exposure amount to seeds is 950 ppm s (= 1.90 mg L^{-1} s), calculated with a temperature of 23.6°C, a pressure at 1013 hPa, and a molecular weight 48. Therefore, the total exposure amounts of O_3 to seeds are 22.8, 68.4, and 114 mg L^{-1} s for plasma irradiation for 1, 3, and 5 min, respectively. The considerations on other RONSs specific to plasma irradiation will be provided in future.

Table 1 shows the percentage of germination (at 12 hours after imbibition) of seeds exposed to different treatments, such as humidification and exposure to plasma irradiation in different combinations. The average percentage of germination under all conditions was 0% at 0 h and over 90% at 24 h (see Table S1), indicating that the maximal germination of lettuce seeds was not affected within 2 days of humidification. The progressive increase in germination percentage at 12 h after imbibition of seeds subjected to humidification only (without plasma irradiation) was observed with increasing the duration of humidification (the percentage of germinated seeds was enhanced by 85 and 128 % after 1 and 2 days of humidification, respectively). Plasma irradiation for 1 min did not have impact on germination at 12 h, while 3 min or prolonged treatment stimulated germination at 12 h (by 39%) in seeds humidified for 1 day. However, this effect was not statistically significant due to the large scatter of results. Negative effects (-31%) of plasma irradiation for 3 and 5 min was observed in seeds humidified for 2 days. The results indicate that the plasma irradiation effects depend on the humidification time. On the other hand, the dependence of the germination characteristics on the time of humidification and plasma irradiation also suggests that an optimum condition for treatment duration can be determined. It is important to note, that the germination percentage at 24 h after imbibition was above 90 % even in seeds irradiated with plasma for 5 min (Table S1). Thus, plasma irradiation did not negatively affect the final germination percentage. A notable finding is the lack of a cancelling effect between humidification and plasma irradiation. The germination rate at 12 h was significantly (156%) increased by humidification for 1 day and plasma irradiation for 3 min, compared to the control (no humidification and no plasma irradiation). The ratio of 3-min plasma irradiation to 0-min irradiation was consistently 1.4 for both 0- and 1-day humidification. Furthermore, the ratio of 1-day humidification to 0-day humidification remained at 1.8 for both 0- and 3-min plasma irradiation. The maximum germination rate of 20.0% at 12 h was not achieved when humidification and plasma irradiation were applied independently, indicating a positive additive effect between humidification and plasma irradiation. Such additive effects were also found for other conditions. We found a significant increase in germination rate at 12 h, compared to the control, for 0.5 day of humidification and 3 min of plasma irradiation (71%), 1 day of humidification and 5 min of plasma irradiation (100%), and 2 days of humidification and 1, 3, and 5 min of plasma irradiation (114%, 56 %, and 56%, respectively).

These results indicate that both the intrinsic effect of humidification and the extrinsic effect of plasma irradiation may be important factors determining the germination kinetics. This result is consistent with the results shown by August et al.[25] who found that stimulation of germination by plasma irradiation in *Arabidopsis thaliana* seeds is much stronger in seeds containing 30 % water compared to seeds with lower (3 or 10 %) water content and concluded that increasing seed water content improves the plasma-triggered dormancy release.

Aiming to gain more information on such effects we estimated the impact of the humidification on the seed water content. Figure 2 shows the dependence of water content in seeds on humidification time. The obtained results revealed that water content monotonically increases from 4.7 % to 10.8% with an increase in the duration of the humidification from 0 day to 2 days. In the first day of humidification, the water content increased linearly. The fitted equation is given by

$$y = 4.0x + 4.8, \quad (R^2 = 0.999) \qquad (1)$$

where y and x is the water content (%) and humidification duration (days), respectively. The water absorption rate from the air with 85 %Rh into seeds was constant until 9 % of water in seeds was achieved. The water absorption rate R_{abs} of early 1 day of humidification is given by

$$R_{\mathrm{abs}} = \frac{\alpha}{100} \times M_{\mathrm{s}}/M_{\mathrm{w}} \qquad (2)$$

where α is a slope of water content per day, 4.0 % day^{-1} from eq. (1) , M_{s} is a mass of a lettuce seed, 0.8 mg/seed[16], and M_{w} is a mass of a H_2O molecule, 18 g/mol. R_{abs} is 1.8 μmol day^{-1} seed^{-1} for 85 %Rh air at 22°C. Thus, the change in sensitivity of germination to plasma irradiation due to humidification (shown in Table 1) may be explained by an increase in the water content of seeds. According to August et al, the increase in water content results in a transition of the cellular cytoplasm from a glassy to a rubbery state followed by a better diffusion of plasma-generated RONS that releases dormancy[25)]. Therefore, examining the effects of plasma irradiation on seeds with changing physiological states while maintaining low moisture content is intriguing.

Besides humidification, we attempted to change the physiological state of seeds by other means, such as seed storage time, which requires long-term experiments to eliminate other effects caused by the moisture absorption. Figure 3 presents the dependence of seed water content on the storage time of seeds. The presented results indicate that seed water content remained constant for 152 days. The estimated mean and standard deviation were 4.7 % and 0.3 %, respectively. Such result indicated that seeds were stored under appropriate conditions to maintain low water content during the storage period.

It is well known that seeds undergo after-ripening and dormancy break during longer storage due to ROS production accompanying the changes in the balance of phytohormones[26),27)]. We carried out the study on the effects of plasma irradiation on lettuce seeds stored for different time durations aiming to estimate the impact of the seed dormancy state on plasma-induced changes in the parameters of germination. The results presented in Figs. 4(a) and 4(b) show how the percentage of germination at 12 and 24 h after imbibition depends on the duration of seed exposure to plasma discharge. As one can see, the differences in plasma effects on germination are more obvious at 12 h (Fig. 4(a)) compared to 24 h after imbibition (Fig. 4(b)). As shown in Fig. 4(a), the percentage of seed germination at 12 h without plasma irradiation (0 min) was 7.8 % at 0 day of storage. It gradually increased with the storage time, reaching a maximum of 46.7% at 39 days, and decreased further increasing the duration of storage. This finding indicates that seed dormancy was broken by storage around 39 days in this study. As shown in Fig. 4(b), germination at 24 h or maximal seed germination for all experimental groups was similarly high and did not fall below 90% for 152 days. The presence of optimal time for the germination percentage at 12 h indicated that the germination rate of seeds decreased after 39 days of storage, most possibly due to seed senescence. Thus, during 152 days of storage, seeds undergo dormancy break and early senescence.

The finding that both control seeds and seeds irradiated with plasma for 1, 3, and 5 min had an optimal germination percentage after 39 days of storage (Fig. 4(a)) indicates that the SDBD plasma had a little effect on the timing of the dormancy break. Seed treatment with plasma for 1 min tended to improve the germination characteristics but only until dormancy break (the mean percentage of germination at 39 days was 1.14 times compared to the control). Longer duration of seed exposure to plasma (3 min) did not help to increase positive plasma effects, and 5 min irradiation even decreased the germination percentage at 12 h after 39 days of storage. These results indicate that the susceptibility of seeds to plasma irradiation varies depending on the physiological state of seeds. In other words, for lettuce seeds, plasma irradiation for 1 min positively affected on the germination rate (estimated by the germination percentage at 12 h) until dormancy break only.

To comprehensively examine the effect of plasma irradiation on germination by the number of days elapsed, we introduced a heat map method. Fig. 5 shows a heat map of the average germination percentage at 12 h after seed imbibition. The heat map was obtained by spline fitting of the germination percentage data. Such analysis revealed that the strongest positive effect of seed irradiation with plasma on germination percentage (a hot spot) appears on day 39 for 1 min treatment duration. Increasing the duration of plasma irradiation above 1 min does not have the apparent effect on the germination percentage. Therefore, we conclude that the dormancy state of lettuce seeds is an important parameter for the effect of plasma irradiation. Since seeds are senescent due to excessive storage[19)], aging is thought to be an additional factor important for the decrease in germination in the storage period after dormancy break in this study.

Early senescent after dormancy break was studied using ESR analysis. Fig. 6 shows typical spectra of ESR signals in the region of g = 1.84 to 2.19 after plasma irradiation for 0, 1, 3 and 5 min for the seeds stored for 82 days. In line with standard practice, ESR analysis is typically conducted after seeds have shown signs of aging and their final germination rate has declined [28)]. Consistent with this, our study initially planned to perform ESR analysis after observing a decrease in the final germination rate. Despite the lettuce seeds maintaining a final germination rate of over 90% at 82 days of storage (and even after 152 days, finally, as shown in Fig. 4), the possibility of early senescence could not be ruled out. Therefore, we decided to initiate ESR analysis after 82 days of storage, earlier than originally planned. A peak appeared at g = 2.00, assigned as the semiquinone radical [12,13)30)]. No new peak appeared after plasma irradiation. This result is in agreement with the previous studies using radish seeds[21)]. The peak at g = 2.00 became larger due to plasma irradiation. The width of negative and positive peaks and the intersection with signal intensity at 0 showed no difference among samples. Consequently, the signal intensity obtained by subtracting the minimum value from the maximum value indicates the number of semiquinone radicals. Fig. 7 shows signal intensity for seeds irradiated for 0-, 1-, 3- and 5-min with plasma (raw data available in Table S3). The signal intensity was 0.97×10^4 without plasma irradiation (0 min), and it increased to 1.30 x 10^4 after 1 min of plasma irradiation, and further increased to 1.63 x 10^4 after 3 min of plasma irradiation. However, with plasma irradiation for 5 min, the intensity was 1.52 x 10^4, which is almost the same as 3 min-plasma irradiation. Fig. 7 also shows the intensity dependency at g = 2.00 on plasma irradiation time for quercetin. This result was obtained by irradiating quercetin powder (ChromaDex; ASB-00017030-100) with plasma. For this, 1 mg of quercetin was placed on a quartz plate yielding density of 1mg / 100 μl and followed by ESR measurement. One sample of quercetin was prepared per condition. Quercetin is one of the typical phenolic compounds found in plants such as lettuce [31)] able to scavenge superoxide anion[32)]. The signal intensities obtained for quercetin powder irradiated for 0, 1, 3, and 5 min with plasma were 1.30 × 10^4, 2.55 × 10^4, 2.77 × 10^4, and 2.77 × 10^4, respectively. This result qualitatively resembles the behavior of lettuce seeds as shown in Fig. 7. Formation of organic radicals from phenolic compounds is responsible for the change of the signal intensity and the results showed that it depends on the duration of plasma irradiation. The possible mechanism involved in increasing intensity at g = 2.00 is that -OH group in hydroquinone $C_6H_4(OH)_2$ in the seeds is converted to -O* by plasma irradiation, yielding semiquinone radical $C_6H_4OHO^*$[33),34)]. Semiquinone radical is further oxidized to the form of quinone $C_6H_4(=O)_2$ that does not contribute the signal intensity of ESR at g = 2.00 [35)]. However, in Fig. 7, a significant decrease in signal intensity with increasing irradiation time is not observed. This can be explained by considering the following equilibrium reaction (3).

$$Q + O_2^{*-} \rightleftarrows SQ^{*-} + O_2 \qquad (3)$$

where Q, O_2^{*-}, and SQ^{*-} show quinone, superoxide, and semiquinone radical, respectively. Therefore, Fig. 7 suggests that quinone increased by plasma irradiation reacts with relatively long-lived O_2^{*-}, and the reverse reaction that returns to semiquinone radicals becomes noticeable especially in the range of plasma irradiation for 3 min or more. Reaction (3) also shows that oxygen molecules, which are more transport friendly than flavonoids, accept electrons from semiquinone radicals. This suggests that oxygen molecules act as carriers in the signal network for propagating the stimulation of plasma irradiation to other tissues in seeds. This may be a unique characteristic of plasma irradiation to dry seeds.

Figure 8 shows the signal intensity ratio on the storage duration. The signal intensity ratio was obtained by normalization based on the signal intensity without plasma irradiation. R_1, R_3, and R_5 show the signal intensity ratios for plasma irradiation duration of 1-, 3-, and 5-min, respectively. After 82 days of storage, R_1 = 1.33, R_3 = 1.67, and R_5 = 1.57, all of which are maximal. After that, although the relative intensity fluctuates, it tends to decrease with storage time (correlation coefficients of R_1, R_2, and R_5 are -0.59, -0.67, and -0.57, respectively). This result suggests that the amount of antioxidant molecules (*e.g.*, glutathione [36)] and vitamin E [37)]) that can non-enzymatically scavenge ROS by becoming semiquinone radicals decreases with the time of storage[18)]. According to S. Yu *et al.*, the number of antioxidant flavonoids in seeds decreases with aging[38)]. Therefore, ESR results indicate that the seeds had started senescence after dormancy break, supporting the result of Fig. 4. At 152 days of storage, R_1 = 1.11, R_2 = 1.14, R_5 = 1.10, which did not fall below the intensity without plasma irradiation but closely approached 1.00. This finding suggests that flavonoids are greatly reduced compared to 82nd day of seed storage.

In summary, we carried out the experimental study on treatment of the lettuce seeds with plasma, focusing on the dependence of plasma effects on germination on the physiological state of seeds that was modified by humidification and storage. The germination percentage of seeds without plasma irradiation varied depending on the time of humidification and storage. Experiments using seeds with humidification showed that germination rate increased with the time of plasma irradiation and humidification, and then decreased. Since the amount of water in the seeds increases with humidification and that leads to faster activation of metabolic seed processes in seeds leading to an increase in the germination rate. The heat map result suggests that controlling the dormancy state by the seed storage time may improve the reproducibility of the plasma irradiation effects. Furthermore, in the case of lettuce seeds and SDBD plasma irradiation, the effect of plasma irradiation was not observed after breaking seed dormancy. ESR measurement showed that seeds were undergoing early senescence in this storage period. These results revealed that the dormancy state of seeds is an important parameter impacting the effects of seed irradiation with plasma.

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Author contributions

T.O. contributed to experiments such as plasma irradiation, the germination test, and ESR measurement, preparing manuscript, and discussion on molecular modification of quercetin radicalization and the seed storage time dependence of germination induction effect by plasma irradiation. T.A. contributed to experiments such as plasma irradiation, seed moisture measurement, germination test, and ESR measurement. S.H. contributed to the experiments such as measurement of O_3 concentration and power consumption. P.A. contributed to discussion on ESR results. K.Kamataki and M.S. contributed to discussion on results including variation analysis of seed moisture content by humidification. N.Y. and N.I. contributed to discussion on quercetin molecular structure changes. Y.I. contributed to experimental methods of humidification and storage of seeds, and discussion on results germination characteristics and early senescence. K.Koga contributed to research design, discussion of results and direction, preparing manuscript. V.M. contributed experimental methods such as ESR, discussion on the results and writing paper.

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Figure Captions

Fig. 1. The ozone concentration at 5 mm below the electrode at 0, 10, 30, 50 s elapsed after plasma generation for 5 s. Marks show the mean values and error bars show standard deviations. The grey area shows the limit of detection of 4 ppm.

Fig. 2. The dependence of seed water content on humidification time.

Fig. 3. The dependence of seed water content on the time of storage.

Fig. 4. The germination percentage at (a) 12 h and (b) 24 h after seed imbibition for seeds treated with plasma discharge for 0 (control), 1, 3, and 5 min at 0, 22, 39, 67, 96, 152 days of storage.

Fig. 5. A heat map of the average germination percentage ate 12 h after imbibition, obtained by spline fitting of germination percentage data. The vertical axis shows the time of plasma irradiation (min) and the horizontal axis shows the time of storage (days). The color bar indicates the germination percentage.

Fig. 6. Typical spectra of ESR signals in the region of g = 1.84 to 2.19 for lettuce seeds without plasma irradiation and with 1-, 3- and 5-min-plasma irradiation for stored for 82 days.

Fig. 7. Signal intensity for lettuce seeds and quercetin exposed for 0, 1, 3 and 5 min to plasma irradiation.

Fig. 8. The dependence of the relative signal intensity in seeds irradiated for 1, 3, and 5 min by plasma normalized by the intensity in seeds without plasma irradiation. The relative intensities R_1, R_3, and R_5 show the plasma irradiation duration for 1, 3, and 5 min, respectively.

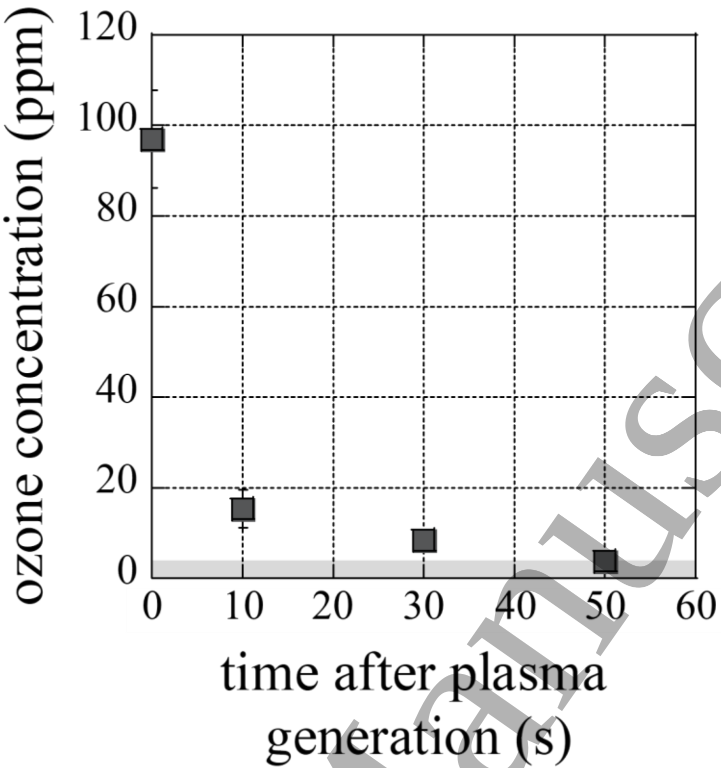
ozone concentration (ppm)
120
100
80
60
40
20
0
0 10 20 30 40 50 60
time after plasma generation (s)

Fig. 1.

Table 1. The germination percentage of lettuce seeds at 12 h after imbibition. The symbols * indicate statistically significant effect of plasma treatment (difference from 0 min plasma irradiation and 0 humidification duration). The symbols # indicate statistically significant difference between non-humidified (0 humidification duration) and humidified groups for the same duration of plasma treatment (the differences were assumed as statistically significant at the level of $p \leq 0.05$). The means of three replicates ±SD are presented.

		Plasma irradiation duration (min)			
		0	1	3	5
Humidification duration (day)	0	7.8 ± 1.57	10.0 ± 2.72	11.1 ± 1.57	7.8 ± 6.85
	0.5	12.2 ± 3.14	12.2 ± 3.14	13.3 ± 0.0*	8.9 ± 1.57
	1	14.4 ± 4.16	11.1 ± 3.14	20.0 ± 2.72**,#	15.6 ± 1.57**
	2	17.8 ± 6.29	16.7 ± 2.72*	12.2 ± 1.57*	12.2 ± 1.57*

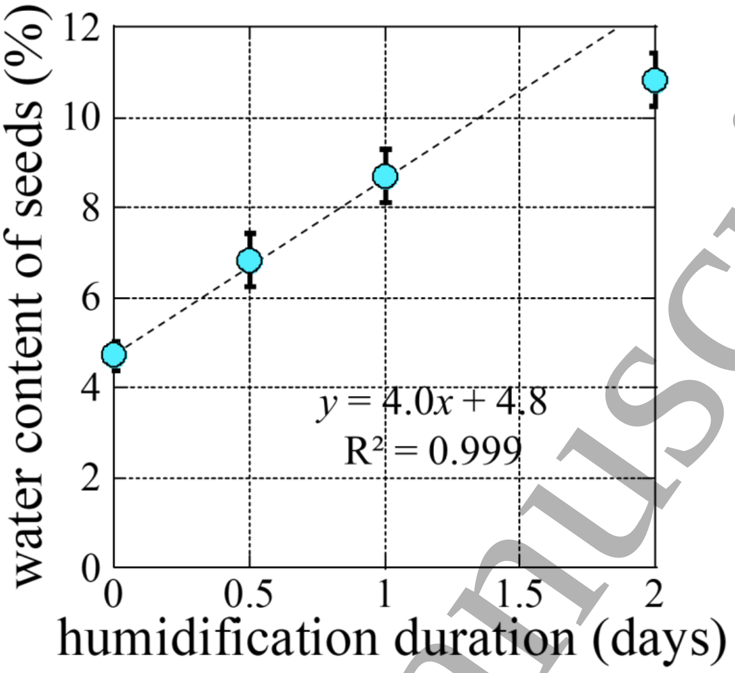
water content of seeds (%)
12
10
8
6
4
2
0
0
0.5
1
1.5
2
humidification duration (days)
y = 4.0x + 4.8
R² = 0.999

Fig. 2.

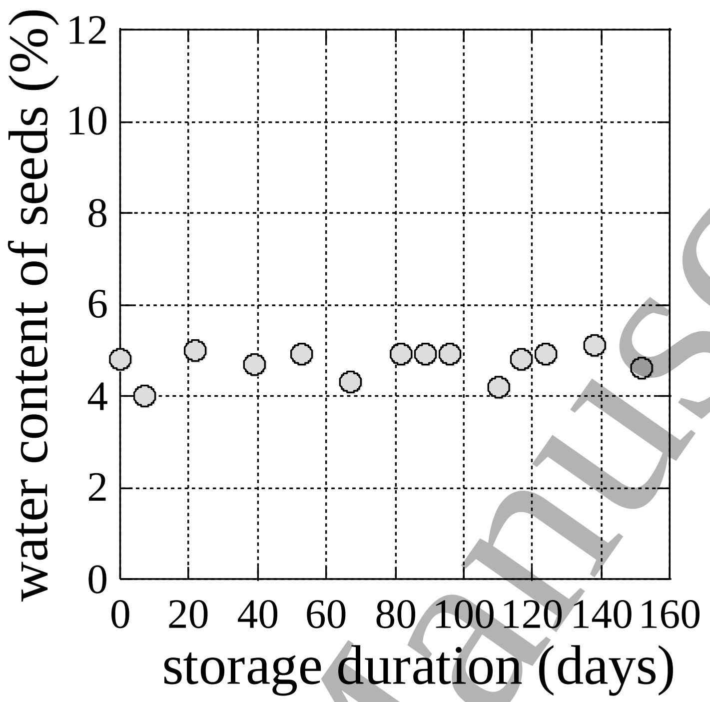
water content of seeds (%)
12
10
8
6
4
2
0
0 20 40 60 80 100 120 140 160
storage duration (days)

Fig. 3.

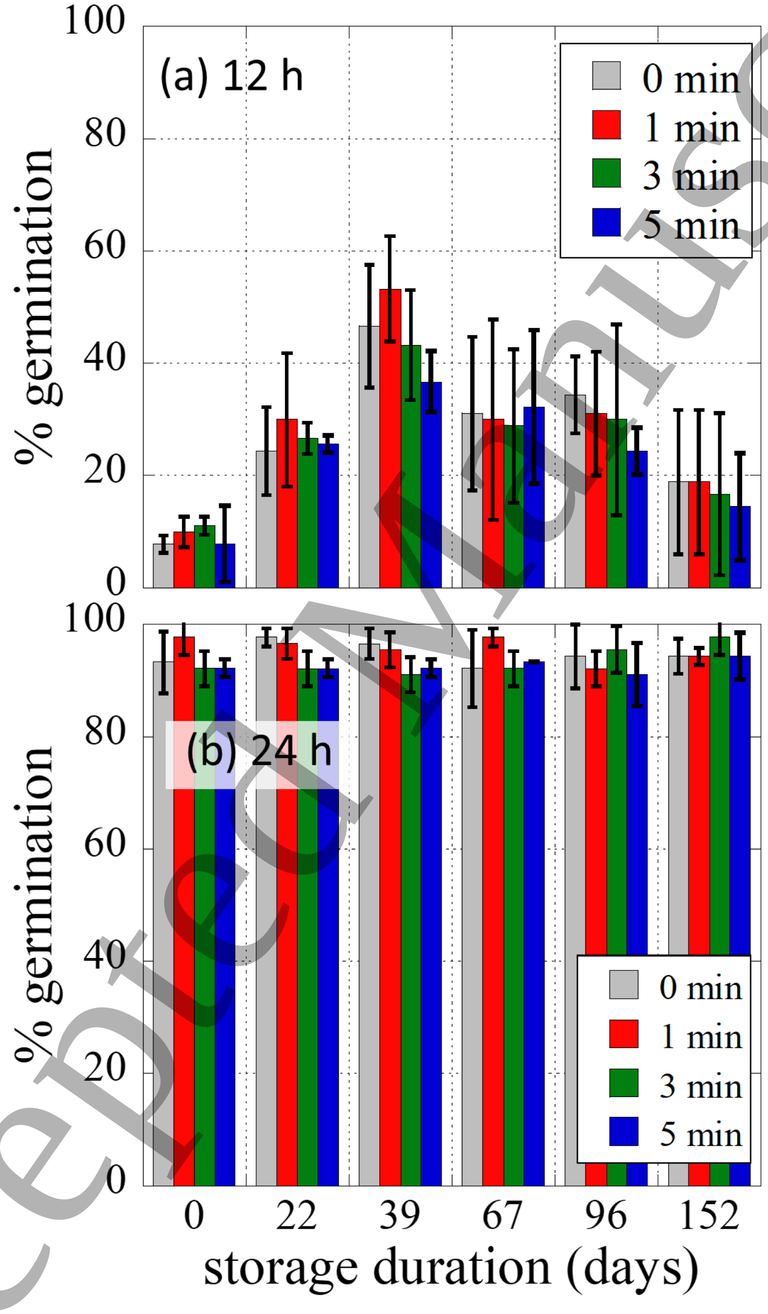
(a) 12 h
(b) 24 h
% germination
100
80
60
40
20
0
0 min
1 min
3 min
5 min
0
22
39
67
96
152
storage duration (days)

Fig. 4.

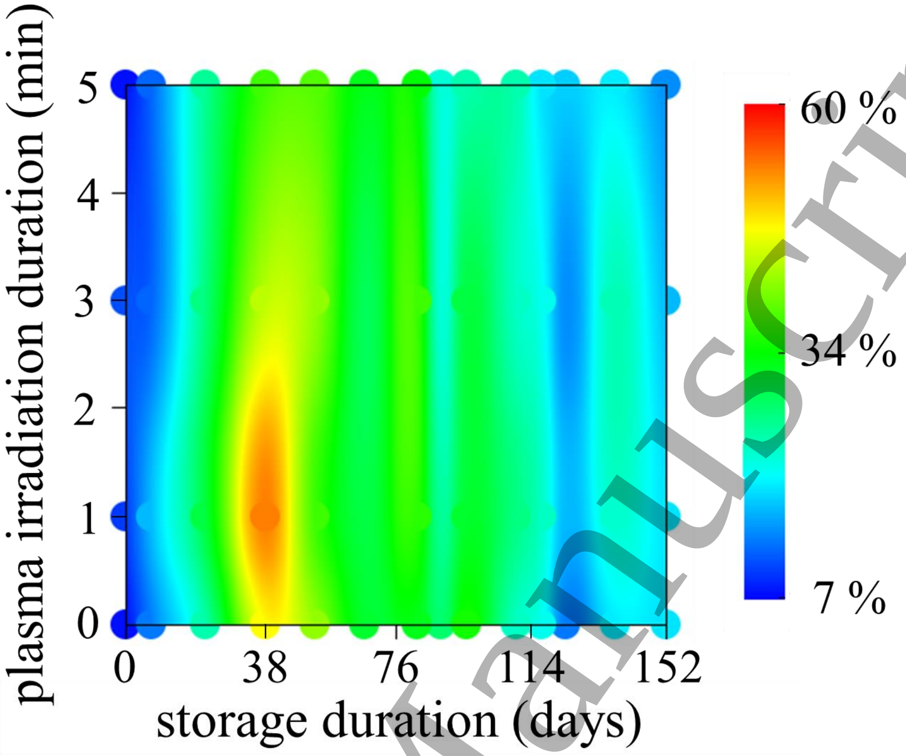
plasma irradiation duration (min)
5
4
3
2
1
0
0
38
76
114
152
storage duration (days)
60 %
34 %
7 %

Fig. 5

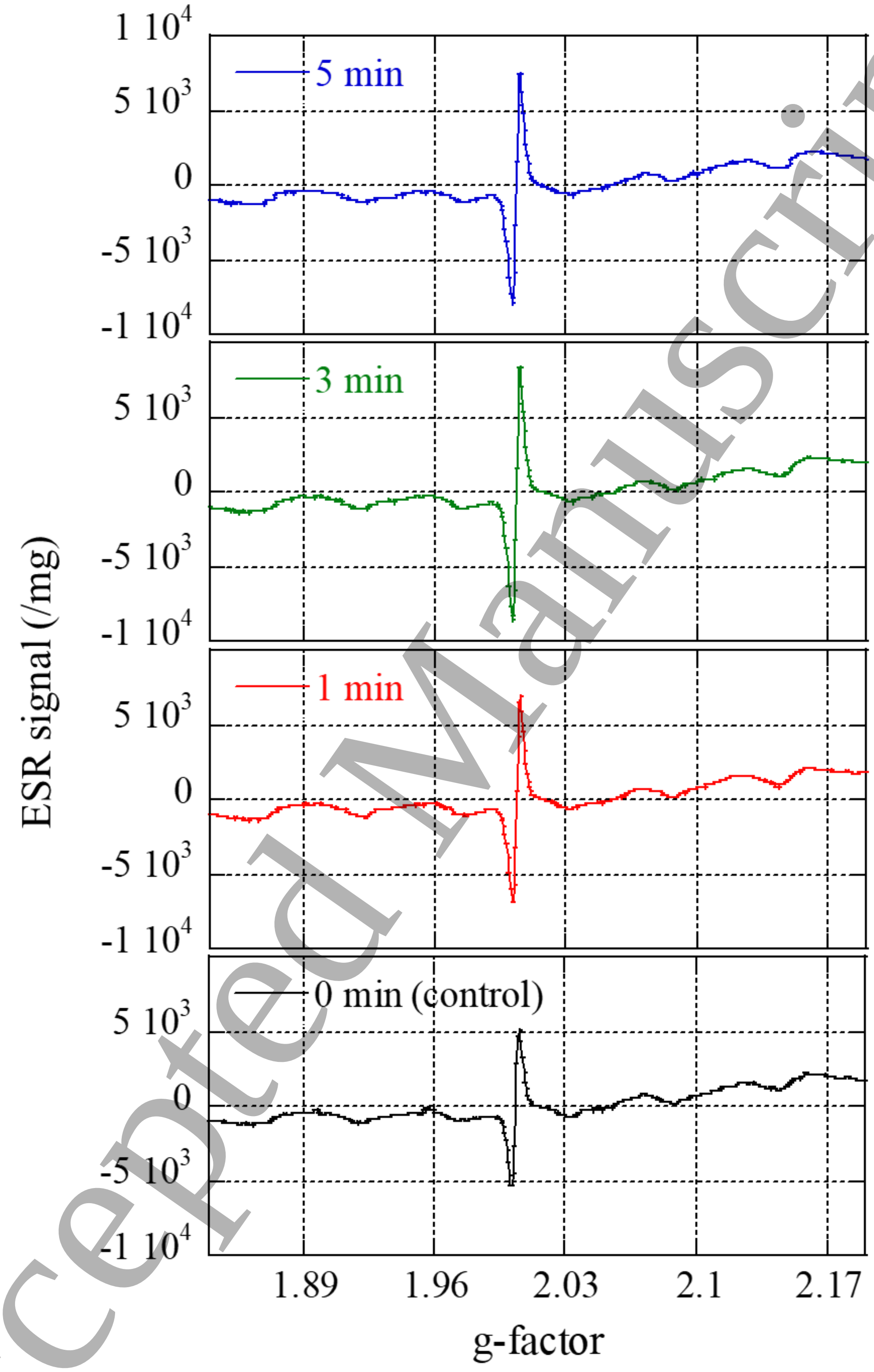
5 min
3 min
1 min
0 min (control)
1 10^4
5 10^3
0
-5 10^3
-1 10^4
ESR signal (/mg)
1.89
1.96
2.03
2.1
2.17
g-factor

Fig. 6

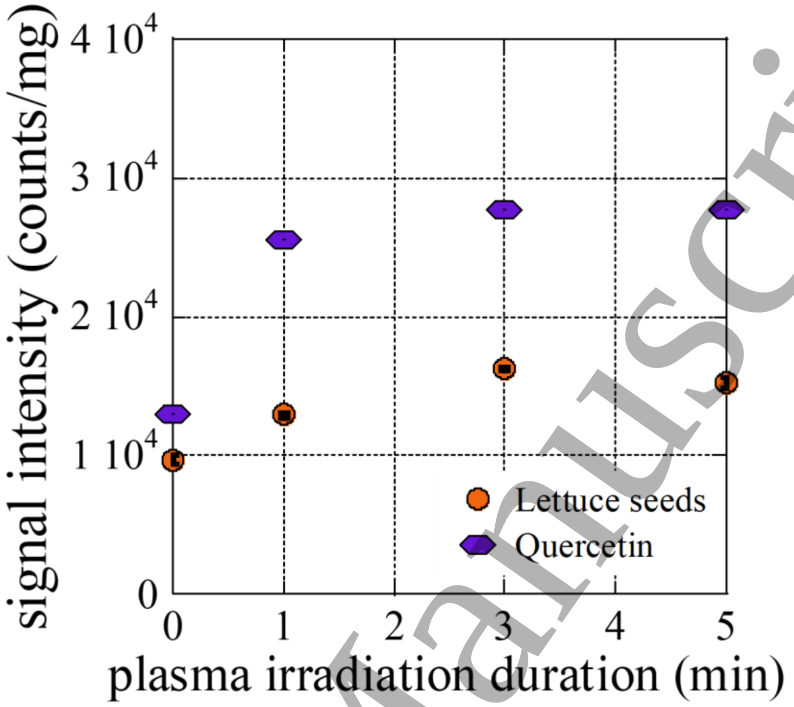
signal intensity (counts/mg)
4 10^4
3 10^4
2 10^4
1 10^4
0
0
1
2
3
4
5
plasma irradiation duration (min)
Lettuce seeds
Quercetin

Fig. 7

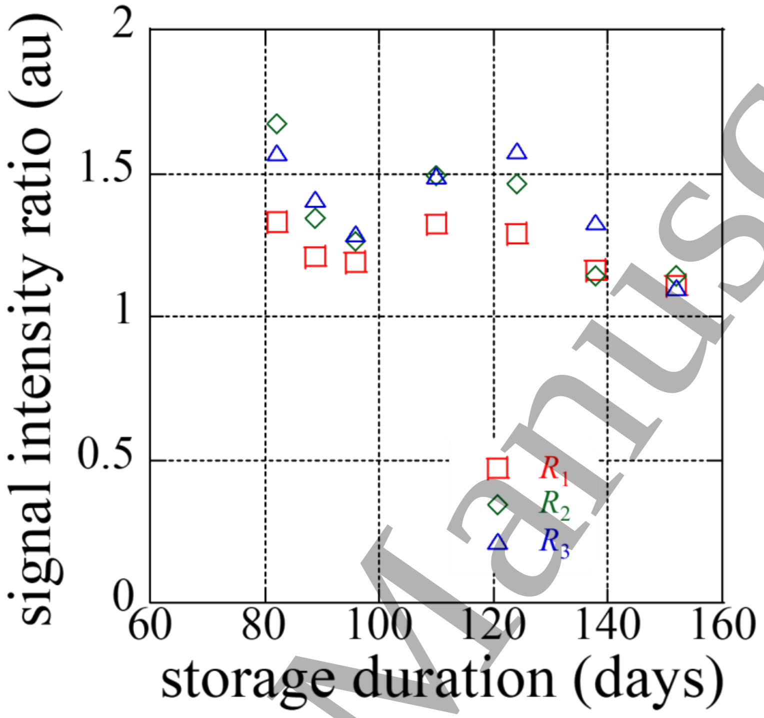
signal intensity ratio (au)
2
1.5
1
0.5
0
60
80
100
120
140
160
storage duration (days)
R_1
R_2
R_3

Fig. 8