Gene expression analysis of *Arabidopsis thaliana* irradiated with oxygen plasma

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Abstract: Growth enhancement are observed pure growth effects by irradiating plasma to leaves that are cells after differentiation. The growth suppression effect such as the reduction of leaf area and suppression of the sites and functions related to glycoprotein production and photosynthesis was observed. Also, the activation of chaperonin which may lead to a growth enhancement effect was observed.

Keywords: oxygen plasma, plant, leaf, growth suppression, gene expression.

1. Introduction

Recently, enhancement of germination and growth, and improvement of antioxidant activity have been found as an effects of active species in plasma on plant cells [1][2]. In order to clarify the enhancement mechanism of biological function by plasma, gene expressions analysis of *Arabidopsis thaliana* seeds irradiated with plasma has been carried out [3]. In the gene expression analysis, the activation of RuBisCO which is an enzyme catalysing the photosynthetic reaction, the energy production pathway leading to the glycolysis system and the TCA (tricarboxylic acid) cycle, Expression of genes related to plant hormone production, activation of antioxidant substances was found to be change. Also, seeds are undifferentiated cells. As a result of the gene expressions analysis, genes involved in differentiation as well as genes of growth enhancement and growth inhibition were similarly detected.

On the other hand, since leaf and stem are consisted of cells after differentiation, gene expressions analysis of leaves is shows effects of promoting or inhibiting the plant growth as a response to plasma. However, it has hardly been investigated that changes in gene expressions when irradiation with plasma on the leaves and stems of the germinated plant. In this study, responses of plant cells after leaves are irradiated with active species in plasma. Plant parts those respond to plasma irradiation are specified and its response mechanism by performing microarray analysis in clarified comparing the results with the case of plasma irradiation to seeds.

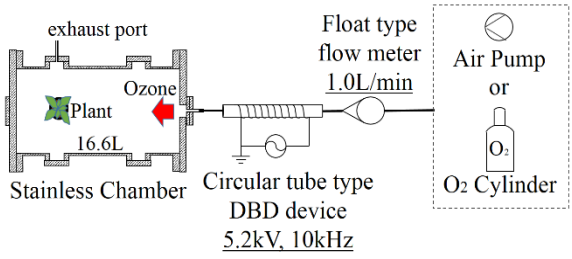

Fig. 1. Schematic of plasma irradiation experiment on plants after germination.

Table 1. Plasma irradiation conditions on leaves.

Description	Gas type	Irradiation period	Number of irradiation
(1) 10s×3	Oxygen	10 seconds	Once a day 3 days
(2) 20s	Oxygen	20 seconds	Once
(3) Air30s	Air	30 seconds	Once

2. Experimental procedure

A schematic diagram of the experimental apparatus is shown in Fig. 1. When the dielectric barrier discharge was generated by a high frequency and high voltage power supply, an atmospheric pressure plasma was generated from air or oxygen gas, and introduced into a processing container made of stainless steel.

For plasma irradiation experiments on leaves and stems, *Arabidopsis thaliana Columbia - 01* grown for one month after seeding was used as a sample plant and was placed in the processing container with soil. Plasma irradiation on the leaves was performed under three conditions; (1) 10s×3, (2) 20s and (3) Air30s. (1) 10s×3 means irradiation oxygen plasma for 10 seconds once a day for 3 days. (2) 20s means irradiation oxygen plasma for 20 seconds once. (3) Air30s means irradiation air plasma for 30 seconds once. Details of the plasma irradiation conditions are shown in Table 1. After irradiation with the plasma, 5 large leaves per one strain are selected and the averaged leaf area is measured.

RNA with the weight of 50 ng was extracted from leaves after 6 hours from the plasma irradiation using RNA reagents, and gene expressions change was analysed.

3. Results

Changes in leaf area after the plasma irradiation were measured, shown in Fig. 2. The area became smaller as compared with the non-irradiated leaves (control). This phenomenon is known as plant ozone disorder, which may be a problem especially with plasma sterilization of agricultural products. Therefore, clarifying mechanism of ozone disorder is indispensable for the development of plasma sterilization technology. Significant gene

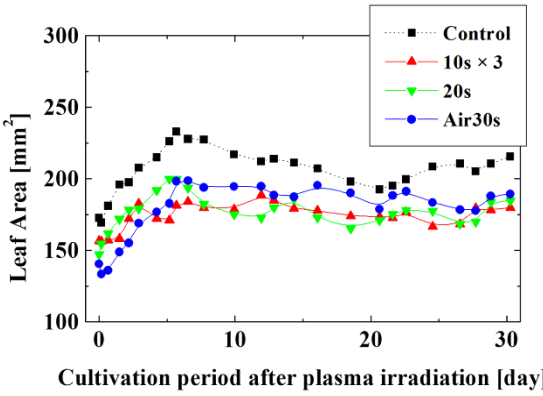

Fig. 2. Leaf area after plasma irradiation for different irradiation conditions.

expressions of the plasma irradiated leaves owing to the plasma irradiation with conditions (1) to (3) are shown below.

(1) 10s×3: Gene expressions those suppress photosynthesis, biosynthesis of glycoprotein, FAD related to energy production, etc. increase. Also, gene expressions those activate biosynthesis of oxidoreductases such as thioredoxin and chaperone increase. Chaperone is proteins for repair of abnormal proteins as heat shock proteins. Gene expressions cluster containing chaperone activated is shown in Table 2.

(2) 20s: Gene expressions those suppresses biosynthesis of photosynthesis, glycoprotein, kinase related to energy production pathway, growth-related EGF and leucine rich repeat increase. Oxidation of glucosinolate, thioredoxin, methionine etc. Expressions of gene that activate enzymes involved in reduction, protein repair pathways increase.

(3) Air30s: Gene expressions those suppresses the biochemistry of glycoprotein, kinase, EGF increases, and gene expressions such as glucosinolate and oxidoreductase in activated.

4. Discussion

The leaf area was observed to be shrunk by plasma irradiation, and the following reaction pathways are considered from the gene expressions results.

Damage to proteins and DNA by reactive oxygen, deterioration of photosynthesis
↓
Decrease in glycoprotein
↓
Decreased ATP production due to suppression of TCA cycle
↓
Cell cycle slowdown, cell number decrease

Obtained gene expressions suggest that leaves respiring through stomata are less resistant to external factors than seeds. When leaves are irradiated by the lower conditions with lower concentrations of ozone, the growth enhancement effect may appear. In addition, with regard to the increased expressions of activation of protein repair enzymes such as chaperone observed in plasma irradiation condition (1), it is possible to lead to growth enhancement by comprehensively repairing damage caused by other than plasma irradiation.

Table 2. Gene expressions cluster containing chaperone activated under plasma irradiation condition (1).

Enrichment Score: 12.1			Count	P_Value
endoplasmic reticulum lumen	RT		15	5.4E-16
Chaperone	RT		25	1.9E-15
Protein processing in endoplasmic reticulum	RT		27	2.4E-14
Endoplasmic reticulum	RT		33	2.0E-11
protein folding	RT		23	6.7E-10
unfolded protein binding	RT		14	7.7E-10

Dependence of the plasma irradiation condition on the gene expressions results in discussed. Under conditions (2) and (3), there was no change in gene expressions of chaperones or heat shock proteins, unlike the change in (1). One of the differences between these conditions is the period from the plasma irradiation to the gene analysis. The hang time duration leads to the expressions of the chaperone etc. which in required about several days until expression. The damage by plasma irradiation is larger in condition (1) than in conditions (2) and (3). Therefore, it is possible that chaperone etc. appeared.

5. Summary

When the plasma irradiates the *Arabidopsis thaliana* leaves, growth suppression was observed by both measurement of leaf area and gene expressions analysis. The sites and functions mainly related to glycoprotein production and photosynthesis are suppressed. The energy production circuit is expected to be suppressed, leading to growth inhibition. Compared with the case where the seeds are irradiated with plasma, the leaves would be more susceptible to external factors because they have stomata and many chlorophylls that are closely related to energy production, and the functional damage is strongly expressed by the oxidation by plasma. Under condition (1), activation of chaperone, which is a protein repair enzyme, was confirmed. This activation may lead to a growth enhancement effect.

References

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