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Effects of Magnesium Deficiency on Induction of Activity of Antioxidative Enzymes*

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Magnesium 결핍이 항산화효소의 활성유도에 미치는 영향*

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ABSTRACT

The influence of deficient(7μM) and suffcient(1000μM) magnesium(Mg) supply on the content of mg, chlorophyll, protein and the activity of superoxide dismutase(SOD), scavengers of superoxide radical(O_2^-), and ascorbate peroxidase(AP), H_2O_2 scavenging enzyme, and glutathione redutase(GR) were studied in pumpkin(*Cucurbita moscata* DUCHESNE) plants over a 11-day period. Over the 11 days period of growth in nutrient solution with sufficient and deficient Mg supply, the contents of Mg, chlorophyll, protein and the activities of the antioxidative enzymes remained more or less constant in Mg-sufficient leaves. In Mg-deficient leaves, the contents of Mg, chlorophyll and protein was seriously decreased with time, however the activities of SOD, AP and GR highly enhanced compared to those of Mg-sufficient. The results indicated the stimulative effect of Mg deficiency on toxic oxygen species and scavenging enzymes in plants

Key word : *Cucurbita moscata* DUCHESNE, Antioxidative enzymes, Mg deficiency, Active oxygen species

INTRODUTION

The oxygen in atmosphere is relatively unreactive, but when it comes into contact with metabolic systems it can be transformed into more reactive forms such as superoxide(O_2^-), hydrogen peroxide(H_2O_2), hydroxy radical(OH ·) and singlet oxygen($^1O_2$). All these forms of oxygen are far more reactive than ground state oxygen and can potentially cause damage to the normally reducing cellular envirnoment. These forms of oxygen are therefore often called active oxygen. Some of these species can cause formation of organic free radicals which, because they are reactive, can interact with a wide range of molecules and cause damage. The response of plants under extreme enviroment are known to be asso-

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ciated with free radical or oxidative damage. The enviroments of extreme are often attributed to air pollutants[24], herbicides[4,29], toxic metals[9], nutrient deficiencies[7], postanoxic injury[26], temperature extremes[21], high photosynthetically active irradiance and ionizing radiation[15]. The resistance of plants against the oxidative stress have an important significance from the point of survival strategy for given species. The toxic oxygen species is generated essentially in chloroplasts and mitochondrias, although the plants grow in normal condition[2,5,33]. However plants have been acquired a kind of defensive ability to overcome the oxidative stress in the course of evolution. The defense mechanisms contain antioxidants such as vitamin C, E, carotenoid and glutathione, and antioxidative enzymes such as superoxide dismutase, ascorbate peroxidase, glutathione reductase and catalase[1,28].

Only a little is known about the effect of mineral nutrient deficiency as a stress factor that affects antioxidative mechanisms in plants[7,8]. Magnesium is a cofactor of chloroplast ribosome (a 70S ribosome) in stroma, and the 70S ribosome is responsible for biosynthesis of large subunits of ribulose 1,5-bisphosphate(RUBP) carboxylase that catalyze CO_2 fixation in photosynthesis[12]. In Mg-deficient chloroplasts, CO_2 fixation can be impaired because chloroplast 70S ribosomes dissociate into 50S and 30S units, which result in inhibition of biosynthesis of RUBP carboxylase. Thus the enhanced O_2 activation can be expected due to the reduction of CO_2 fixation. Also in nonstressed chloroplasts, photoreduction of O_2 is estimated to be between 5 to 27% of the total electron flow[2,5]. Photogeneration of toxic O_2 species can be intensified when plants grown at a high light intensity are exposed to an environmental stress that impairs the utilization of absorbed light energy for pohotosynthetic CO_2 fixation[2,18].

Photoreduction of molecular O_2 in chloroplasts leads to the production of superoxide anion radical($O_2^{\cdot -}$) and H_2O_2[3,31]. Superoxide anion radicals, H_2O_2, and their derivativies ie, hydroxy radical($OH\cdot$) and singlet oxygen($^1O_2$) are highly toxic, resulting in destructive effects on the functional and structual integrity of chloroplasts[10,16]. As protection mechanism against these toxic O_2 species, chloroplasts are equipped with several antioxidants and defense enzymes as above. SOD, mostly localized in chloroplasts in leaves[19], catalyze the dismutation of $O_2^{\cdot -}$ to H_2O_2 and O_2, and the changed H_2O_2 is converted into O_2 and H_2O. As catalase is not present in chloroplasts[3,26], the conversion of H_2O_2 in these organelles is mediated by a H_2O_2 scavenging system with the involvement of AP, dehydroascorbate reductase and GR[12,13]. In many instances, activities of SOD and enzymes involved in the H_2O_2 scavenging pathway are increased in response to environmental stress factor such as drought[34], chilling[32], and treatment with hyperbaric O_2 level[11] and air pollutants[35].

In this study, we studied the alteration of activities of $O_2^{\cdot -}$ and H_2O_2 scavenging enzymes in pumpkin plants subjected to Mg-sufficient and deficient condition as a part of understanding for the process of acclimation of plants to environmental stress.

MATERIALS AND METHODS

Plant Growth

Pumpkin(*Cucurbita moschata* Duchesne) plants were grown under controlled environmental conditions(light/dark regimes 14/10h at 27/22℃, relative humidity 65-75%) with light intensities of 20,000 lux provided by fluorescent and incandescent light.

Seeds were germinatied in vermiculate saturated with water. After 7 days, the seedlings were

transferred to nutrient solutions. The nutrient solution was made by slightly modified composition of nutrient solution described elsewhere[8]. Composition of the nutrient solution used was as follows(M) : 0.88×10^{-3} K_2SO_4 ; 2×10^{-3} KCl ; 1×10^{-5} H_3BO_3 ; 4×10^{-5} FeEDTA ; 1×10^{-6} $MnSO_4$; 1×10^{-6} $ZnSO_4$; 1×10^{-7} $CuSO_4$; 1×10^{-8}(NH4)6MoO_{24} ; 1×10^{-3} Mg(sufficiency) or 0.7×10^{-5} Mg(deficiency) as $MgSO_4$.

Plants were harvested after 5 to 11 days growth in nutrient solution. At harvest, primary leaves were separated and photographed and dried at 105℃ during 24 hours for determination of Mg concentration. For analysis of enzyme activities determination of chlorophyll contents, primary leaves were harvested, frozen in liquid nitrogen, and stored at -70℃ prior to analysis.

Enzyme Determinations

About 0.5g leaf material without the main midrib was ground with liquid nitrogen and homogenized with 5mℓ of 100mM potassium phosphate buffer(pH 7.0) containing 5mM EDTA, 1mM AsA and 20% PVP(w/v) in a mortar and pestle. The homogenate was centrifuged at 12,000g for 20min. The supernatant was kept in icy water and used for determination of enzyme activities. All enzyme activities were measured at 25℃ in a final volume of 1mℓ using various aliquots of the supernatants(25-50μℓ for ascorbate peroxidase ; 100μℓ for glutathione reductase ; and 25 - 100μℓ for superoxide dismutase).

Activity of AP was measured according to Nakano and Asada[31] by monitoring the rate of ascorbate oxidation at 290nm(E=2.8mMcm^{-1}). The reaction mixture consists of 50mM potassium phosphate buffer(pH7.0), 0.1mM H_2O_2, 0.5mM ascorbate, and the enzyme aliquot. Glutathione reductase was assayed according to the method of Foyer and Halliwell[12] by following the decrease in absorbance at 340nm due to NADPH oxidation(E=6.2mMcm^{-1}). The reaction mixture contained 100mM potassium phosphate buffer(pH 7.8), 3mM $MgCl_2$, 0.5mM GSSG, 0.2mM NADPH, and the enzyme aliquot. SOD was measured by the method as described by McCord and Fridovich[23]. Prior to assay, crude enzyme was dialyzed for 24hours at 2℃. The assay mixture comprised of 50mM potassium phosphate buffer(pH7.8), 0.1mM xanthine monosodium salt, 10mM cytochrome C, and an aliquot of xanthine oxidase(XOD) that required to cause 0.04 increment of the absorbance at 550nm arised by reduction of cytochrome C during 120 second, and the enzyme aliquot. One unit of SOD activity was defined as the amount of enzyme to decrease 50% of absorbance that increased by XOD addition.

Analytical Methods

The Mg concentrations were determined by inductively coupled plasma(ICP) spectrometer after drying samples at 105℃, followed by grinding in a mortar and pestle and extracting in 1N HCl for 12hours[22]. Concentrations of chlorophyll(a + b) were measured after extraction with dimethyl sulfoxide at 60℃ for 12hours as described by Hiscox and Israelstam[17]. Protein concentration was determined by the method of Bradford[6].

RESULTS AND DISCUSSION

Sufficient Mg supply increased dry matter production significantly in roots and shoots over 11 days of growth. However, at low Mg supply, dry weight increments were slight(data not shown). As compared with Mg-sufficient plants, the Mg concentrations in the leaves of Mg-deficient plants were significantly lower during the growing period, and were about 1/14 in 5 days to that of sufficient Mg supply(Fig. 1). And in the leaves of Mg sufficient, Mg concen-

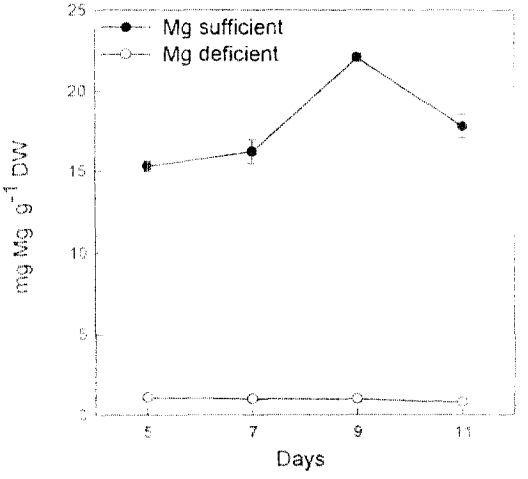

Fig. 1. Concentration of Mg in primary leaves of pumpkin plants over 11 days of growth in nutrient solutions with sufficient(1,000μM) and deficient(7μM) Mg supply. Each data point represents the mean of three replicates. Bars indicate SE.

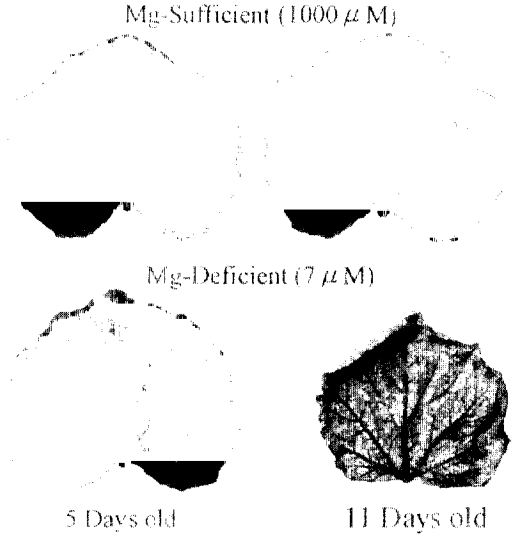

Fig. 2. Leaf damage of pumpkin plants grown in Mg-sufficient and deficient nutrient solutions.

tration was increased gradually with time.

Chlorosis in leaves arised after 5 days that pumpkin plants was grown in nutrient solution with deficient(7μM) Mg supply(Fig. 2). The chlorosis was proceeded rapidly with time and

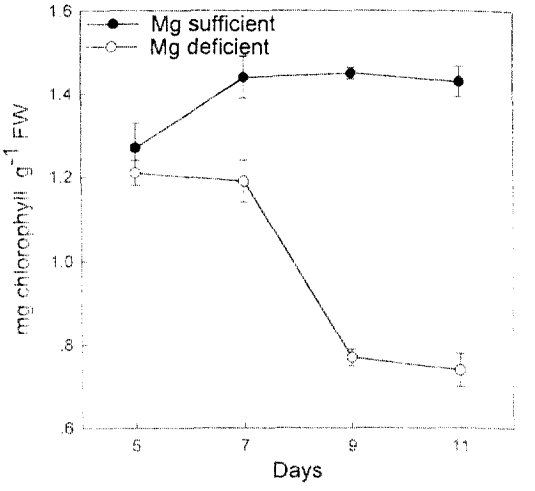

Fig. 3. Concentrations of chlorophyll in primary leaves of pumpkin plants over 11 days of growth in nutrient solutions with sufficient (1,000μM) and deficient(7μM) Mg supply. Each data point represents the mean of three replicates. Bars indicate SE.

most distinguished in Mg-deficient leaves after 11 days. Chlorophyll contents were little affected by two different Mg concentration after 5 days growth(Fig. 3). Thereafter, chlorophyll content in Mg-sufficient leaves was increased slightly. However, in those that were Mg-deficient, decrease in chlorophyll content was especially distinguished at the period from 7 days to 9 days.

The decrease in protein concentration became more remarkable in Mg-deficient leaves with time(Fig. 4). Protein concentration in Mg-sufficient leaves decreased gradually to 84% in plants after 11days but that in Mg-deficient leaves decreased rapidly to 32% compared to those grown for 5 days in nutrient solution, respectively. The rate of reduction of protein was most remarkable within 5 to 7 days in Mg-deficient leaves. Since degradation of chlorophyll was most stroking at the period from 7 to 9 days(Fig. 3), decomposition of protein seems to rapidly preceded that of chlorophyll.

Effects of Mg supply on antioxidative enzyme

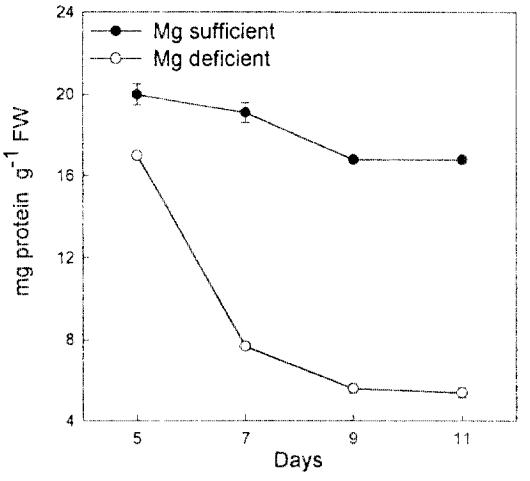

Fig. 4. Changes of protein contents in primary leaves of pumpkin plants over 11 days of growth in nutrient solutions with sufficient (1,000μM) and deficient(7μM) Mg supply. Each data point represents the mean of three replicates. Bars indicate SE.

activities are shown in Fig. 5. For a given harvest, Mg levels shown in Fig. 1 influenced the enzyme activites in Mg-deficient leaves. The activity of AP, GR and particularly SOD(Fig. 5) increased as a function of time and process of Mg-deficient symptom development(Fig. 2, 3, 4). Even as early as day 5, when first visual symptoms of chlorosis became evident(Fig. 2), activites of AP, GR and SOD were about 2.1, 1.4 and 1.4 times higher, respectively, in leaves of Mg-deficient compared with sufficient plants(Fig. 5). For a given period, the activites of antioxidative enzymes by day 9 were highest : the activites of AP, GR and SOD were higher by times of about 5.2, 3.4 and 18.1, respectively, in Mg-deficient leaves than in Mg-sufficient leaves.

With the onset of visual Mg deficiency symptoms, there is a rapid increase in the activities of antioxidative enzymes against toxic O_2 species. The increase in the activities of AP, GR and SOD in Mg-deficient plants occur with distinct declines in chlorophyll and protein concen-

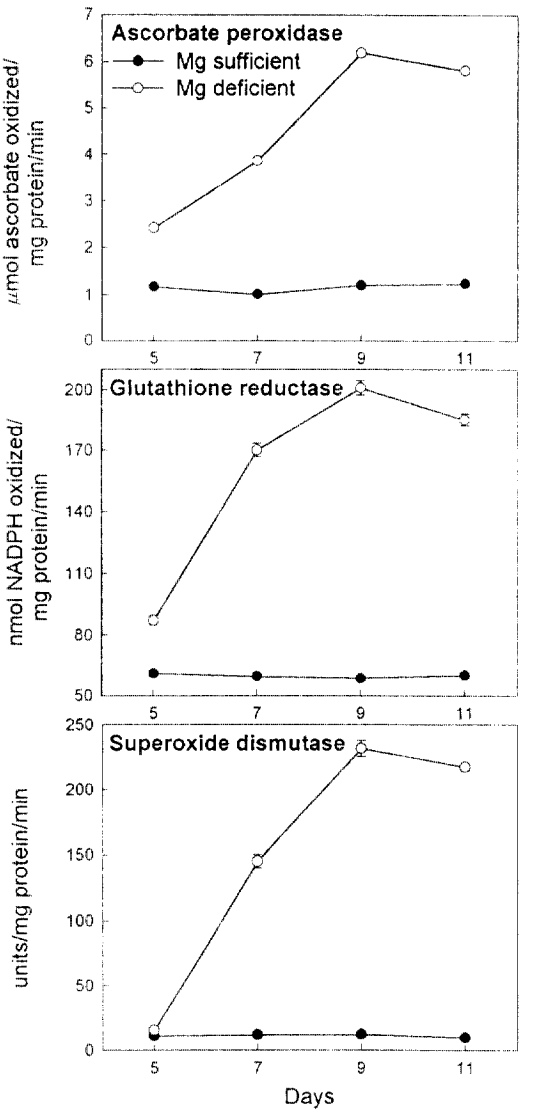

Fig. 5. Activities of ascorbate peroxidase, glutathione reductase and superoxide dismutase in primary leaves of pumpkin plants over 11 days of growth in nutrient solutions with sufficient(1,000μM) and deficient(7μM) Mg supply. Each data point represents the mean of three replicates. Bars indicate SE.

trations(Fig. 3, 4, 5), indicating that elevated levels of antioxidative enzymes can be considered as physiological defense response of plants to Mg deficiency. Commonly, it is known to that active oxygen species attacks to biomole-

cules such as lipids, proteins, nucleic acids and carbohydrates *etc* in plant cells and caused eventually to cell death[1]. We assumed that enhancement of antioxidative defense mechanism also arised in the plants which was peroxidizing by attack of Mg deficiency-induced toxic O_2 species, since the protein and chlorophyll concentration decreased rapidly with time, whereas the activities of AP, GR and SOD increase greatly in Mg deficient plants(Fig. 3, 4, 5).

The enhancements in activities of antioxidative enzymes by Mg deficiency most probably take place in chloroplasts where SOD[21] and the H_2O_2 detoxifying enzymic cycle[41] are predominantly located. From the results presented here, It seems that photooxidation of thylakoid constituents are a major contributing factor in development of Mg deficiency chlorosis. Furthermore, the differences between plant species in the expression of visual Mg deficiency symptoms may be related to constitutive differences in generation or detoxification of harmful O_2 species in the chloroplasts(in preparation). The enlargement in antioxidative defense enzymes indicates Mg deficiency-induced enhancement of production of toxic O_2 species, particularly superoxide and H_2O_2. It is well reported that such protective mechanism is induced under environmental stress conditions can produce toxic O_2 species as mentioned elsewhere[1,30].

Increased capacity for the detoxification of toxic O_2 species was known to that increase tolerance or resistance of plants to related stress such as chilling[20], ozone[35] and herbicide paraquat[33]. Recently, we found that Mg-deficient leaves are much more resistant to paraquat and oxyfluorfen than Mg-sufficient leaves, and newly proteins induced in different plant species by Mg deficiency(in preparation). Experiments are now underway in our laboratory to sequence the proteins that are induced in various plant species by Mg deficiency.

摘　　要

有害한 活性酸素種들을 생성하는 환경 stress에 대한 식물의 適應過程을 파악하기 위한 일환으로서, 호박을 식물재료로 공시하고, Mg이 缺乏된 營養液에서 재배하여 酸化的 stress 조건을 부여하였을 때, 나타나는 過酸化의 피해정도와 抗酸化酵素들의 活性變動을 조사하였다.

충분한 양의 Mg이 공급된 영양액에서 재배된 호박의 잎에서는 Mg, 葉綠素, 蛋白質 含量 및 抗酸化酵素들의 活性이 다소 증가 되었거나 거의 일정하게 유지되었다. 그러나, Mg이 缺乏된 營養液에서 재배된 호박의 잎에서는 Mg, 葉綠素 및 蛋白質의 含量은 현저히 감소된 반면, 抗酸化酵素인 AP, GR 및 SOD의 活性이 크게 증가되었다. 본 연구에서 얻어진 결과들은 식물에서 Mg의 缺乏으로 인하여 해로운 活性酸素種이 생성되며 동시에 이들活性酸素種의 有害한 작용을 消去하기 위한 抗酸化酵素의 活性도 誘導되는 것을 시사한다.

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