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Effects of Sulfur Dioxide on Pigments, Protein Content and Photosystem Ⅱ Activity of Barley and Corn Leaves

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보리와 옥수수 잎의 色素, 蛋白質 含量 및 光系Ⅱ活性에 미치는 SO_2의 影響

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ABSTRACT

This investigation was carried out to clarify the changes of pigments and soluble protein, and photosystem Ⅱ activity in the leaves of barley (SO_2-sensitive) and corn (SO_2-resistant) seedlings induced by the SO_2 fumigation (10, 50 ppm). The pH changes of the leaf extract, the content of sulfite and sulfate, the activities of catalase, peroxidase, and polyphenoloxidase were compared in the leaves of barley and corn seedlings induced by SO_2 fumigation. The results are summarized as follows: An appreciable effect of pH change of leaf extract by SO_2 fumigation was observed in barley leaves (pH 6.10 to 5.18), but only a small change occurred in corn leaves (pH 5.66 to 5.50). The same pattern of pH changes was recorded when the solution of 0.2 N HCl was added to leaf extract, providing lower buffering capacity of the barley leaves than corn leaves. After 2 hours of exposure to 10 ppm SO_2, the contents of SO_3^{2-} and SO_4^{2-} were increased in barley leaves, while only SO_4^{2-} increased in corn leaves. After fumigation with 10 ppm SO_2 for 2 hours, barley leaves showed significant decreases in activities of catalase, to 17% peroxidase, to 58%, and polyphenoloxidase, to 88%. Corn leaves showed increases in activities of peroxidase, to 136%, and polyphenoloxidase, to 128%. Absorption spectra of pigments obtained from SO_2-fumigated leaves were gradually decreased with the fumigation time increases, but the decrease was more significant in barley leaves. Fumigation with 50 ppm SO_2 for 2 hours induced the greatest decomposition in carotenoid, followed by chlorophyll *a* and then chlorophyll *b* in barley leaves. The ratio of chlorophyll *a*/*b* was decreased from 4.1 to 3.6 in barley leaves, but in corn leaves it was maintained almost a constant level (4.9—4.8). The rate of decomposition of chlorophyll and caro-

tenoid in corn leaves was very slow than those in the barley leaves. Fumigation with 50 ppm SO_2 for 2 hours, decreased the protein content of barley leaves to 59%, and that of corn leaves to 89%, and the extent of decrease in protein content was greater than that of pigments in barley and corn leaves. The rate of DCIP(dichlorophenol indophenol) photoreduction in SO_2- fumigated leaves was decreased to 18 and 67% in barley and corn leaves, respectively. However, DCIP photoreduction was considerably recovered about 32 and 92% with the addition of DPC(diphenylcarbazide) as an exogenous electron donor in barley and corn leaves, respectively.

INTRODUCTION

Sulfur dioxide is a major atmospheric contaminant resulting primarily from the combustion of sulfur-containing fossil fuels. Study of the effects of sulfur dioxide on plants was first initiated in the late nineteenth century. Sulfur dioxide has a special attention among the air pollutants since sulfur is one of the essential plant nutrients(Thomas *et al.*, 1944; Schiff and Hodson, 1973). The sulfur generally has been absorbed in the form of SO_2 from the atmosphere if it is in a very low concentration. However, when the concentration of SO_2 absorbed beyond a certain critical level, it is often noted that the photosynthesis, respiration, and other fundamental cellular processes are impaired. Indeed the high concentration of SO_2 exsists for a certain time which causes the irreversible injury and leads to dead. For plants, thus the harmful effect of SO_2 is discernible (Thomas *et al.*, 1943; Reinert *et al.*, 1969; Tingey *et al.*, 1971).

The phytotoxic behaviour of SO_2 has been described by a number of studies during the last decades (Hill and Thomas, 1933; Setterstrom *et al.*, 1938; Taniyama, 1972; Matsuoka, 1978). It has been shown that the phytotoxic effect to the plant was variable from the concentration and lengh of exposure (Setterstrom and Zimmerman, 1939; Thomas, 1951; Ziegler, 1975; Malhotra and Hocking, 1976). Among the SO_2-effect chlorosis and necrosis are the most prominent phenomena which derived from the breakdown of photosynthetic pigments localized in the thylakoid membranes. Rao and Le Blanc(1965) has reported that laboratory exposure of lichens to lethal doses of SO_2 resulted in the breakdown of chlorophyll into phaeophytin and Mg^{2+} ions. Gilbert(1970) studied the effect of extended exposure to very low SO_2 concentration on sensitive and resistant species of lichens and mosses sampled from relatively unpolluted areas. Puckett *et al.*(1973) suggested that the toxic effect of an aqueous SO_2 on lichens related to its oxidation-reduction properties which induced apparently the destruction of chlorophyll at low pH condition.

When plants are fumigated with SO_2, the toxicant entering leaf tissue is incorporated into thylakoid membranes preferentially (Ziegler, 1977; Garsed and Read, 1977) and induces swelling of thylakoid membranes (Wellburn, *et al.* 1972) or disintegration of the membranes (Malhotra, 1976). Therefore, the process of photosynthesis would be

greatly affected.

Plant species and varieties, and even individuals of the same species, may vary considerably in their sensitivity or tolerance to SO_2 (O'Gara, 1922; Le Blanc *et al.* 1972; Genys and Heggertad *et al.*, 1973; Jensen *et al.*, 1976; Linzon, 1978). Several investigators have attempted to quantify these differences (O'Gara, 1922: Miller *et al.*, 1974). It is known, for example, that among agricultural species alfalfa and barley are sensitive to SO_2, whereas corn and potatoes are relatively resistant (O'Gara, 1922; Thomas *et al.*, 1950). The visual symptoms of SO_2 toxicity on different species of vegetation have been well described (Linzon, 1978: Malhotra and Blauel, 1980).

Thomas and Hill(1935) showed that the degree of injury in alfalfa plants, which had been subjected to varying amounts of light and moisture in the presence of SO_2, was highly correlated with the amount of SO_2 absorbed. Thomas *et al.*(1950) speculated that the species differences in resistance to SO_2 were mainly due to the differences in the rate of absorption of SO_2. On the contrary, in most cases, resistance is not related to the amount of SO_2 absorbed; for example, tolerant perennial rye grass from a polluted site in Lancashire actually has absorbed more SO_2 than the sensitive crops (Hällgren, 1978). It is known that the tolerance in these plants, as is commonly believed, owe not to its exclusion of SO_2 from the cells and organelles.

Most of the SO_2 absorbed by leaves enters through stomata and dissolves in the moist surfaces of mesophyll cells (Thomas *et al.*, 1950). The resulting sulfurous acid (H_2SO_3) dissociates into H^+, HSO_3^- and SO_3^{2-}. Thomas *et al.* (1950), as well as Ziegler(1975) showed that the acidic effect of the H_2SO_3 caused by low fumigation intensity can be buffered. However, the buffer action of the leaves gradually decreases.

It may be assumed that part of the SO_3^{2-} formed by SO_2 uptake probably will be oxidized directly according to the redox conditions prevailing in the cells(Ziegler, 1975) SO_3^{2-} is oxidized to SO_4^{2-} in plant leaf, and this ability has been correlated with the resistance to its toxicity (Miller and Xerikos, 1979). Aerobic oxidation of SO_2^{2-} is known to be enhanced by ultraviolet radiation, by catalysts such as metal ions, and by several enzymes, including several oxidase (Asada and Kiso, 1973). Therefore, plant cells having absorbed SO_2 will experience an accumulation of HSO_3^-, SO_3^{2-} and SO_4^{2-}. The three sulfur anions do affect physiological functions such as photosynthetic electron transport (Silvius *et al.*, 1976; Shimazaki *et al.*, 1979), photophosphorylation(Asada *et al.*, 1968; Ryrie and Jagendorf, 1971; Silvius *et al.*, 1975) and CO_2 fixation (Puckett *et al.*, 1973; Ziegler, 1972, 73; Ziegler and Libera, 1975).

This study was carried out to investigate the changes of pigments and protein content, and photosystem Ⅱ activity in the leaves of barley (SO_2-sensitive) and corn (SO_2-resistant) seedlings induced by SO_2 fumigation. To clarify the differences in susceptibility to SO, the pH changes of the leaf extract, the content of sulfite and sulfate, the

activities of catalase, peroxidase, and polyphenoloxidase were detected in the leaves of barley and corn seedings.

MATERIALS AND METHODS

Plant materials. Barley (*Hordeum vulgare* L.) and corn (*Zea mays* L.) seeds were thoroughly washed and soaked in distilled water for 12 hours. They were sowed in the mixture of soil and sand(3 : 1) in plastic pots (28×22×7 cm), which has been placed under natural light conditions. Hoagland solution was applied every 3 days as a nutrients. Ten days old seedings were utilized for the laboratory analysis.

SO_2 fumigation. Ten days old barley and corn seedings were fumigated with 10 ppm or 50 ppm SO_2 in a fumigation box which was kept at 22±2° C, relative humidity 75± 3%, and illumination. The fumigation box (70×50×52 cm) were constructed by the transparent acrylic sheets, SO_2 was prepared by adding H_2SO_4 into $NaHSO_3$ in a closed system of fumigation box. SO_2 concentration have tested by U2-DS SO_2 Ultra Portable Analyzer. The fluorescent lamp with a light intensity of 20,000 lux illuminated just above the leaf canopy.

Measurement of pH change. Ten grams of leaf samples were homogenized in 100 ml of distilled water at 2° C for 2 min. The homogenate was filtered through four layers of gauze. The pH of leaf extracts was measured with Toa pH meter at 20° C. For measurement of buffer capacity, 0.2 N HCl was added to leaf extract.

Determination of sulfite and sulfate. Five grams of leaf samples were homogenized in 200 ml of distilled water at 2° C for 2 min. After the homogenate had been filtered through four layers of gauze, the filtrate was centrifuged at 10,000 *g* for 10 min at 2° C. The supernatant was used for the analysis of sulfite and sulfate. The content of sulfite was measured with standard potassium iodide-iodate titrant (Rand *et al.*, 1975), and of sulfate by turbidimetric methed (Rand *et al.*, 1975). Neutralized sulfate with $BaCl_2$ was determined spectrophotometrically at 420 nm. Anhydrous Na_2SO_4 was used as a standard curve.

Measurement of catalase, peroxidase, and polypheneloxidase activities. The leaf samples, weighing 200 mg, were homogenized with 10 ml of ice-cold 0.1 M phosphate buffer (pH 7.0) with a mortar and pestle. The homogenate was centrifuged at 17,000 *g* for 15 min at 2° C. The supernatant was used for the enzyme assay. The activity of catalase as well as peroxidase was assayed by the method of Chance and Maehly(1955) with a slight modification. Catalase assay, based on the breakdown of H_2O_2, was quantified spectrophotometrically at 240 nm. The reaction medium contained 0.1 M phosphate buffer (pH 7.0), 0.88 M H_2O_2, and 1 ml of enzyme extract in 3.65 ml. Peroxidase activity, based on the oxidation of pyrogallol in the presence of H_2O_2, was measured. The reaction

mixture contained 0.1 M phosphate buffer (pH 7.0), 50 mM pyrogallol, 0.88 M H_2O_2, and 0.2 ml of enzyme extract in 3.8 ml. The amount of purpurogallin formed was determined spectrophotometrically at 420 nm. Reaction mixture for polyphenoloxidase activity consists of the same assay mixture as that of peroxidase without H_2O_2. The amount of the purpurogallin formed was measured at 420 nm. Catalase, peroxidase and poly phenoloxidase activities were expressed as units of activity/min/g fresh weight by using the following formula.

$$\frac{\Delta OD/min \times dilution\ of\ enzyme}{g\ fresh\ weight\ of\ tissue\ sample} = unit\ of\ activity$$

Determination of pigment content. Primary leaves were cut from the tip in the shape of disc, which is 5 mm in diameter. Pigments were extracted from leaf discs with dimethyl sulfoxide (DMSO) by the method of Hiscox and Israelstam (1979). Ten discs of leaf in 5 ml of DMSO were incubated at 65°C in a water bath for 20 min. The amount of chlorophylls and carotenoid in the DMSO extract were determined by the methods of Arnon(1949) and Liaaen-Jensen and Jensen (1971), respectively. Absorption spectra were recorded with a Shimadzu UV-190 Double-Beam Spectrophotometer.

Determination of total soluble protein. Ten leaf discs were ground with 2 ml of ice-cold 0.01 M phosphate buffer(pH 7.8) in a mortar and pestle, The homogenate was centrifuged at 26,000 *g* for 10 min at 2° C and supernatant protein was made by the method of Bradford (1976). Protein content was measured spectrophotometrically at 595 nm. BSA was used as the standard curve.

Measurement of photosystem II activity. Five grams of leaf samples were homogenized for 15 sec in 50 ml of ice-cold 0.05 M phosphate buffer (pH 7.8) containing 0.02 M sucrose and 0.01 M NaCl at 2° C. After the homogenate had been filtered through eight layers of gauze, the filtrate was centrifuged at 5,000 *g* for 5 min at 2° C. The pellet was resuspended in the preparation medium. Preparation was performed at 2° C in darkness and chloroplasts were stored in ice. Chlorophyll was determined in 80% acetone by the method of Arnon (1949). The rate of 2,6-dichlorophenolindophenol(DCIP) photoreduction was determined by following the absorbance changes at 600 nm, using a Bausch and Lomb Spectronic 20 Spectrophotometer. The reaction mixture for DCIP photoreduction contained 0.01 M phosphate buffer (pH 7.8), 0.01 M NaCl, 50 μM DCIP and 20 μg chlorophyll as chloroplasts in 3 ml. A actinic light was supplied by a 500 watt tungsten lamp after passage through a 7 cm layer of water. The light intensity was 30,000 lux on the surface of reaction mixture. The concentration of exogenous electron donors such as manganeous chloride($MnCl_2$) and diphenylcarbazide(DPC) when they are used, were 1 mM and 0.5 mM, respectively. Measurements were performed at room temperature at one minute interval for 3 minutes.

RESULTS AND DISCUSSION

Changes of pH. Table 1 shows the pH changes of leaf extract from barley and corn by SO_2 fumigation. After 2 hours exposure to 10 ppm SO_2, the pH of leaf extract was reduced from 6.10 to 6.05 in barley leaves, and maintained a constant level of 5.66 in corn leaves. The pH changes of leaf extract by SO_2 fumigation at 50 ppm for 2 hours showed some different characteristics in both barley and corn.

Table 1. Changes in pH of barley and corn leaf extract with SO_2 fumigation

SO_2 fumigation		pH	
Concentration	Time(min)	Barley	Corn
10 ppm	0	6.10	5.66
	60	6.03	5.66
	120	6.05	5.66
50 ppm	0	6.01	5.66
	60	5.18	5.48
	120	5.18	5.50

An appreciable effect (from 6.10 to 5.18 of pH) was observed in barley leaves but only a small change (from 5.66 to 5.50 of pH) occurred in corn leaves. The same pattern of pH change was recorded when the solution of 0.2 N HCl was added to leaf extract unexposed by SO_2(Fig. 1). It is supposed that the SO_2, penetrating chiefly through the stomata, is first dissolved in the tissue during formation of H_2SO_3. The dissolution of H_2SO_3 proceeds gradually. Thomas *et al.* (1944) have shown that the acidic effect of SO_2 can, to some extent, be buffered in the plant leaf, presumably by plant leaf proteins. The pH change is usually considered to be slow(Thomas *et al.*, 1944), The leaf extract of barley and corn leaves exposed to 10 ppm SO_2 for 2 hours have the same buffer capacity as SO_2-unexposed leaves. However, Grill and Haertl (1972) and Grill *et al.* (1975) have reported that homogenates of leaves exposed to SO_2 have a lower buffer capacity than do unexposed ones. This result indicates that corn plant has a better buffer

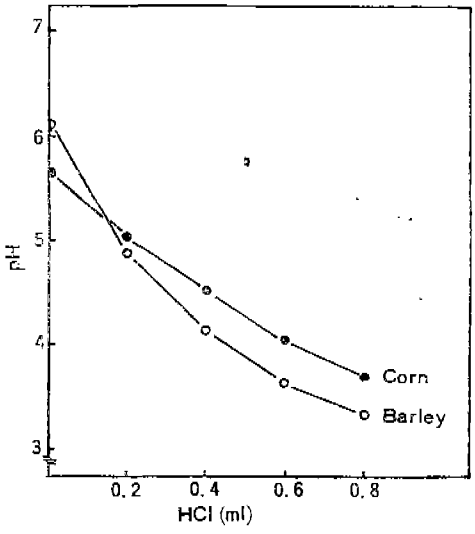

Fig. 1. Changes in pH of barley and corn leaf extract with 0.2N HCl solution.

Table 2. Changes in sulfite and sulfate content of barley and corn leaves with SO_2 fumigation

Fumigation		Sulfite		Sulfate	
Concentration	Time	Barley	Corn	Barley	Corn
	(min)		(μg/g. fr. wt.)		
	0	111	178	94	32
10 ppm	60	125	178	94	44
	120	164	176	107	46

capacity and is able to buffer more acid than do barley plant.

Contents of sulfite and sulfate. Table 2 shows the effects of SO_2 fumigation on the sulfite and sulfate content in barley and corn leaves. After 2 hours of exposure to 10 ppm SO_2, SO_3^{2-} and SO_4^{2-} was increased to 148% and 114% in barley leaves, whereas SO_3^{2-} maintained almost a constant level and SO_4^{2-} was increased to 144% in corn leaves (Fig. 2). In corn leaves, the SO_2 absorbed was mostly oxidized to sulfate, which was much less toxic than sulfite. Thomas *et al.* (1950) reported sulfite is approximately thirty times more toxic than its oxidation product, sulfate, which has been shown to accumulate with other sulfur containing compounds in plant tissues.

It is reported that SO_3^{2-} is oxidized to SO_4^{2-} in the plant leaf, and this ability has been correlated with resistance to toxicity (Miller and Xerikos, 1979; Sugahara *et al.*, 1980). According to Miller and Xerikos (1979), it is suggested the differential capacities of soybean to metabolize toxic sulfite to the less toxic sulfate may be related to differences in SO_2 sensitivity. Therefore, it is supposed that corn leaves make rapid conversion of sulfite to sulfate during the SO_2 treatment period than do barley leaves.

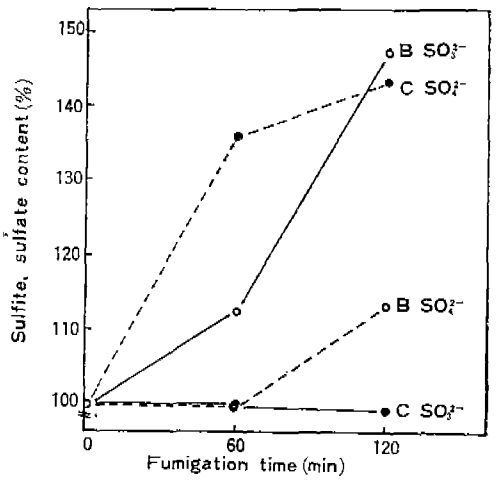

Fig. 2. Changes in sulfite and sulfate content of barley and corn leaves with 10 ppm SO_2 fumigation(B, Barley; C, Corn).

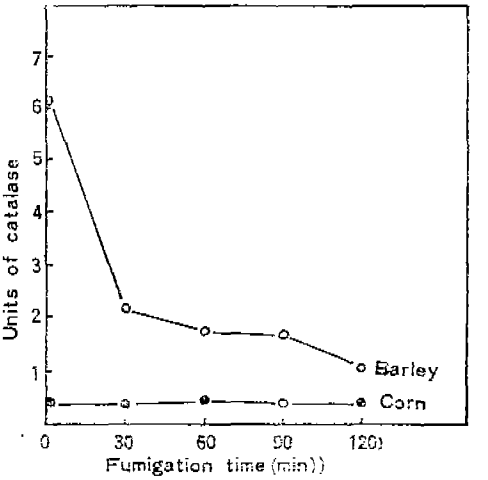

Fig. 3. Changes in catalase activity of barley and corn leaves with 10ppm SO_2 fumigation.

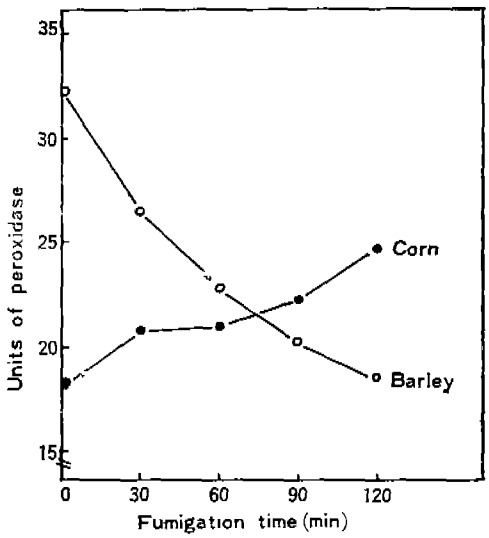

Fig. 4. Changes in peroxidase activity of barley and corn leaves with 10 ppm SO_2 fumigation.

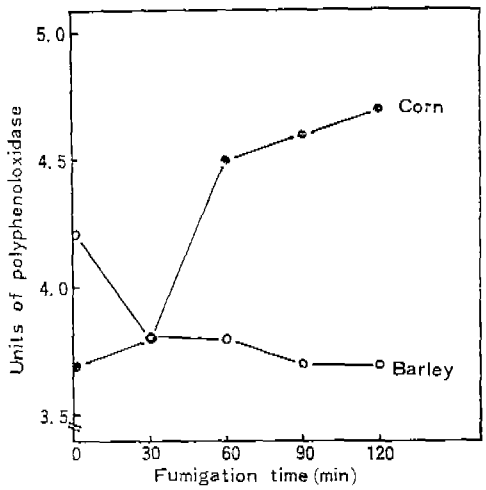

Fig. 5. Changes in polyphenoloxidase activity of barley and corn leaves with 10 ppm SO_2 fumigation.

Changes of catalase, peroxidase, and polyphenoloxidase activities. As shown in Fig. 3, catalase activity was decreased rapidly in barley leaves by 10 ppm SO_2 fumigation for 30 minutes. The activity of corn leaves was extremly low and not changed. Hydrogen peroxide produced through photorespiration is broken down by catalase in peroxisome. It is showed that C_4 plant, corn, has very low photorespiration (Chollet and Ogren, 1975). Therefore, it is supposed that catalase activity, catalyzing the breakdown of H_2O_2, is very low in corn leaves.

Peroxidase and polyphenoloxidase activities were gradually inhibited with 10 ppm SO_2 fumigation period in barley leaves, whereas in corn leaves increased with same exposure (Figs. 4 and 5). SO_2 fumigation inactivated catalase, to 17%, peroxidase, to 58%, and polyphenoloxidase, to 88% in barley leaves (Fig. 6). While, the activities of peroxidase was increased to 136% and polyphenoloxidase 128% in corn leaves (Fig. 7).

Bailey and Cole (1959) reported that SO_3^{2-} is capable of inactivating many enzyme systems by splitting their disulfide linkages. The action of SO_2 is supposed to involve a direct disruption of the enzyme structure, a direct effect on catalytic site, and an indirect effect on cofactors.

Catalase and peroxidase may serve to protect the plants against hydrogen peroxide or may have a broad function in the oxidation of organic molecules. If catalase and peroxidase activities were inhibited by sulfur dioxide in tissues, the tissues would be killed by the accumulation of hydrogen peroxide with active oxygen. Therefore, it was thought that the decrease of catalase and peroxidase activities by SO_2 might be able to increase

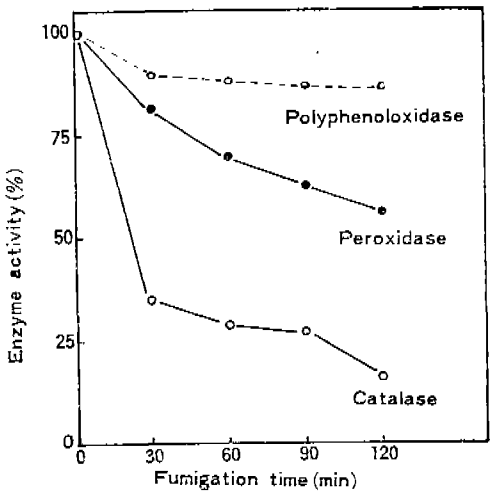

Fig. 6. Changes in catalase, peroxidase and polyphenoloxidase activity of barley leaves with 10 ppm SO_2 fumigation.

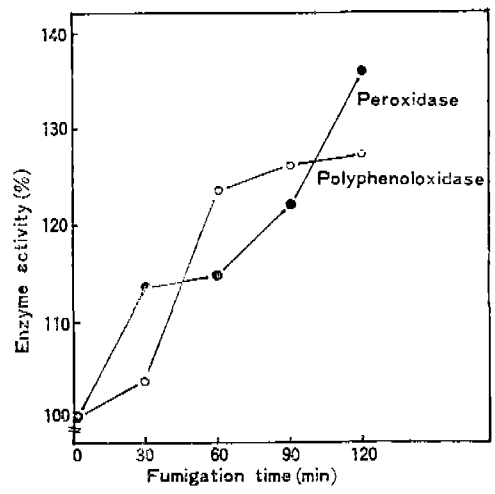

Fig. 7. Changes in peroxidase and polyphenoloxidase activity of corn leaves with 10 ppm SO_2 fumigation.

the accumulation of hydrogen peroxide in cells, which causes the injury of barley leaves.

Changes of pigments. Figs. 8 and 9 show the absorption spectra of pigments obtained from barley and corn leaves treated with 50 ppm SO_2 fumigation. The decrease of absorption occurred both in the red and blue regions by SO_2. Absorption spectra of pigments

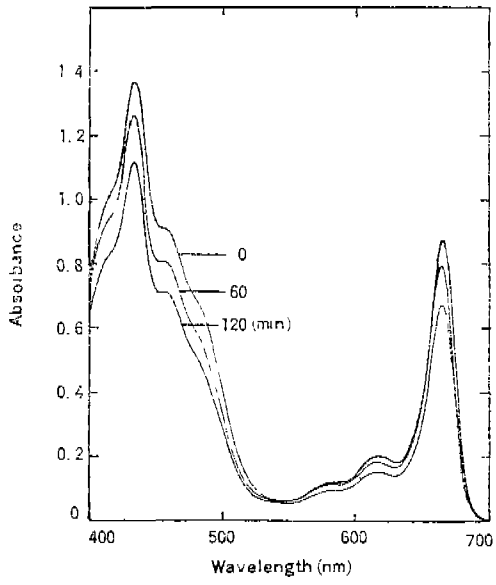

Fig. 8. Effect of 50 ppm SO_2 fumigation on absorption spectra of pigments in barley leaves.

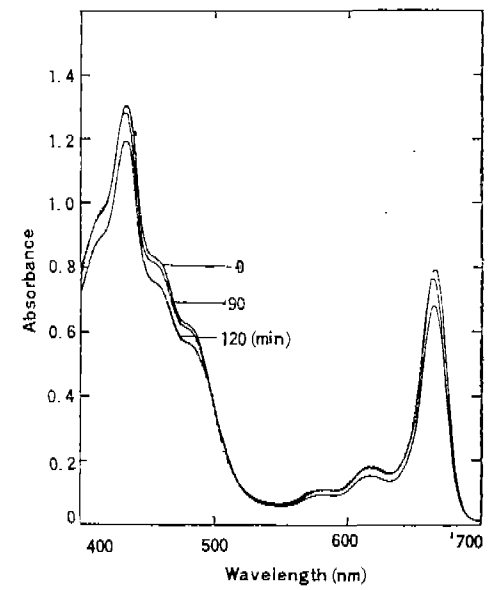

Fig. 9. Effect of 50 ppm SO_2 fumigation on absorption spectra of pigments in corn leaves.

Table 3. Changes in pigment content of barley and corn leaves with 50 ppm SO_2 fumigation

	Fumigation Time(min)	Pigment contents				
		Chl. T	Chl. *a*	Chl. *b*	*a*/*b*	Carotenoid
		($\mu g/cm^2$)				
Barley	0	31.4	25.2	6.2	4.1	6.8
	60	28.9	23.0	6.0	3.8	5.9
	120	24.9	19.5	5.4	3.6	5.1
Corn	0	27.8	23.1	4.7	4.9	6.2
	60	27.2	22.5	4.7	4.8	6.2
	120	24.2	20.0	4.2	4.8	5.7

from SO_2-fumigated leaves were gradually decreased depending on the fumigation periods, but the decrease was more rapid in barley leaves than in corn leaves.

The effects of 50 ppm SO_2 fumigation on pigments content of barley and corn leaves are shown in Table 3. Chlorophylls and carotenoid were gradually broken down, but more significant in barley leaves than in corn leaves. Carotenoid was more rapidly destroyed than chlorophylls in barley leaves, while in corn leaves destruction of carotenoid was less than that of chlorophylls(Fig. 10). Chlorophyll *a* was broken down rapidly, but chlorophyll *b* was slowly degraded in barley and corn leaves(Fig. 11). Chlorophyll *a*/*b* ratio was decreased from 4.1 to 3.6 in barley leaves, but it was maintained almost a constant level from 4.9 to 4.8 in corn leaves(Table 3). Generally, chlorophyll *a* app-

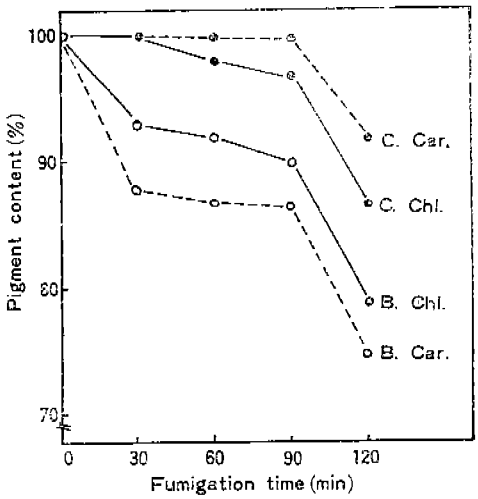

Fig. 10. Changes in total chlorophyll and carotenoid content of barley and corn leaves with 50ppm SO_2 fumigation. (Chl., Chlorophyll; Car., Carotenoid)

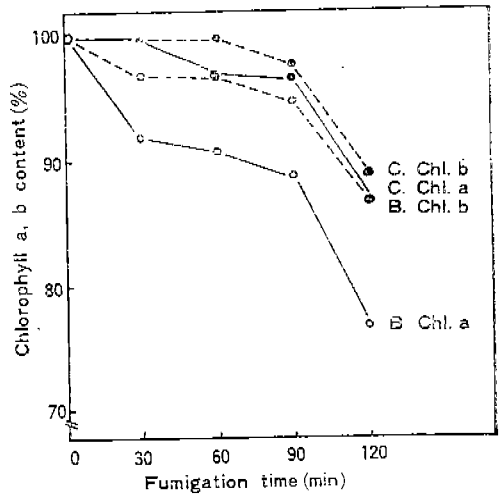

Fig. 11. Changes in chlorophyll *a* and *b* content of barley and corn leaves with 50 ppm SO_2 fumigation.

eared to be more susceptible to SO_2 attack than chlorophyll *b*. The susceptibility of chlorophyll *a* to SO_2 agree well with the early report of other works (Rao and Le Blanc, 1965; Shimazaki *et al.*, 1980).

According to Rao and Le Blanc(1965), chlorophylls are converted to phaeophytins by acid substances, whereby Mg^{2+} is split off and replaced by atoms of hydrogen. Recently, it was demonstrated that the effect of SO_2 on the pigment-breakdown and photosynthesis was not a function of increased acidity (Malhotra, 1977). It is suggested that chlorophyll destruction caused by SO_2 is due to O_2^- production by the reaction of sulfite with chlorophyll under illumination (Peiser and Yang, 1977). Therefore, it may be suggested that chlorophyll *a*/*b* ratio may be a convenient evaluation of plant damage caused by SO_2. In addition, it was also reported that chlorophyll *a* was more sensitive to O_2^- than chlorophyll *b* (Peiser and Yang, 1978).

Changes of total soluble protein. Changes in soluble protein content of barley and corn leaves exposed with SO_2 are shown in Table 4. After 2 hours exposure to 10 and 50 ppm, protein content was reduced to 82 and 59% in barley leaves, and to 96 and 89% in corn leaves, respectively. A significant decrease in leaf soluble protein was observed in the barley leaves exposured to 50 ppm SO_2, but in corn leaves the effect was less appreciable (Fig. 12).

Table 4. Changes in soluble protein content of barley and corn leaves with SO_2 fumigation

Fumigation		Protein content	
Conc.	Time(min)	Barley	Corn
		($\mu g/cm^2$)	
	0	545	534
10 ppm	60	461	517
	120	449	512
	0	556	447
50 ppm	60	350	414
	120	327	369

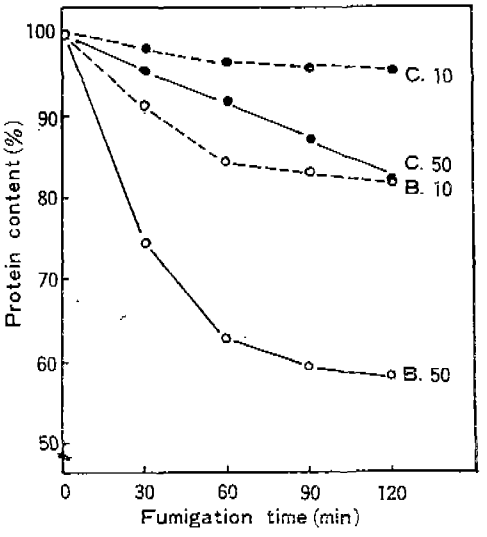

Fig. 12. Changes in total soluble protein of barley and corn leaves with SO_2 fumigation(10, 10ppm; 50, 50ppm).

It was reported that SO_3^{2-} causes a splitting of the protein, probably due to the decomposition of disulfide proteins through the cleavage of S-S bonds in polypeptides (Bailey and Cole, 1959). Cecil and Wake have pointed out that certain disulfide bonds in proteins are readily broken(e.g., cystine), whereas others are highly resistant(Ziegler, 1975). Since the structures and functions of several proteins are highly dependent on the integrity of the disulfide bonds, breakage of

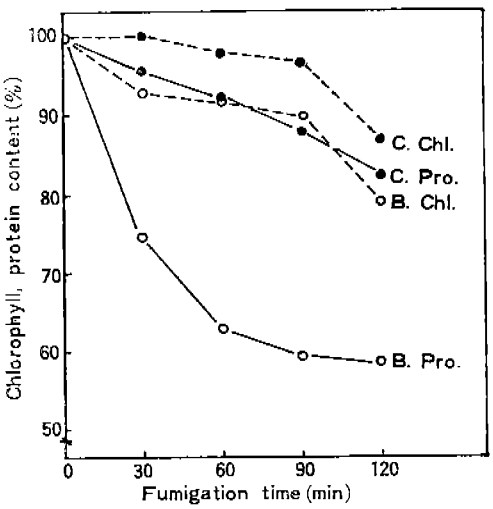

Fig. 13. Changes in chlorophyll and protein content of barley and corn leaves with 50 ppm SO_2 fumigation(Chl., Chlorophyll; Pro., Protein).

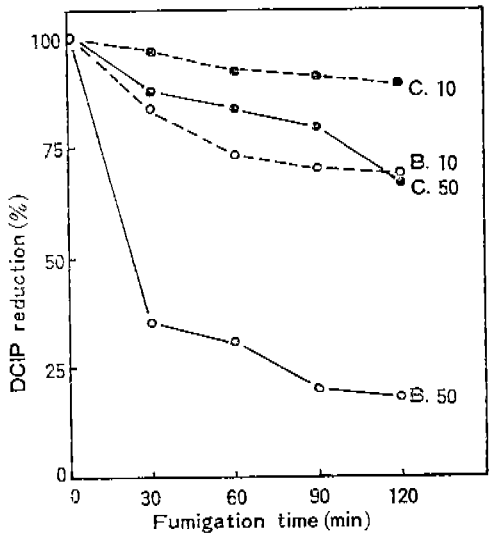

Fig. 14. Changes in DCIP photoreduction of barley and corn leaves with SO_2 fumigation.

these bonds should gradually deactivate several enzymes and alter membrane proteins. With 50 ppm SO_2 treatment for 2 hours, the content of chlorophylls and protein was reduced to 79 and 59% in barley leaves, and to 87 and 83% in corn leaves, respectively. The loss of protein content was much more than that of chlorophyll in barley and corn leaves(Fig. 13). A recent study by Miszulski and Ziegler(1979) indicated that SO_2 fumigation accelerated the thiol groups in thylakoid and the acceleration was greater under illumination than in darkness.

Changes of photosystem II activity. The rate of DCIP photoreduction in chloroplasts isolated from SO_2-fumigated barley and corn leaves is shown in Table 5. After 2 hours exposure to 10 and 50 ppm of SO_2, the rate of DCIP photoreduction was inhibited to 69 and 18% in barley leaves and to 90 and 67% in corn leaves, respectively(Fig. 14). This inhibition maybe due to certain toxic substances formed by SO_2 in leaves and released in the medium during the chloroplast isolation procedure, or due to irreversible damage of reaction components during SO_2 fumigation.

Table 5. Changes in DCIP photoreduction of barley and corn leaves with SO_2 fumigation

Fumigation		DCIP photoreduction			
Conc.	Time(min)	Barley	DPC	Corn	DPC
		(μmoles/mg chl·hr)			
	0	106.3		64.4	
10 ppm	60	78.7		59.3	
	120	72.9		57.8	
	0	90.9		73.7	
50 ppm	60	29.2	47.5	62.2	90.1
	120	16.7	29.2	49.1	68.0

Chloroplasts isolated from non-fumigated leaves were incubated in the supernatant obtained from SO_2-fumigated leaves. After 10min at 0°C in this supernatant, no essen-

tial inhibitory action on DCIP photoreduction was observed(Table 6). Furthermore, the inactivation of PS II activity caused by SO_2 fumigation was not removed by washing it with 10mM phosphate buffer(pH 7.8). These results indicated that SO_2 fumigation did not produce any substance inhibitory to the DCIP photoreduction but induced irreversible damage to the PS II system during the fumigation.

Table 6. Effect of supernatants obtained from SO_2-fumigated leaves on DCIP photoreduction

Incubation supernatant	DCIP photoreduction	
	Barley	Corn
	(μmoles/mg chl·hr)	
Non-fumigated	99.2	85.1
Fumigated	98.5	85.9

Fumigation was performed at 50ppm SO_2 for 1 hour. The rate of DCIP photoreduction in chloroplasts isolated from fumigated leaves was 18.8 and 73.6μ moles/mg chl/hr in barley and corn, respectively.

The rate of DCIP photoreduction inhibited by SO_2 could be recovered by addition of DPC, an artificial electron donor, for photosystem II(Table 5, Figs. 15 and 16). But $MnCl_2$ of 1 mM, an electron donor for photosystem II, showed no effect.

The H^+ produced by SO_2 fumigation may make lower the cytoplasmic pH. When chloroplasts were incubated in an acidic pH, the oxidizing side of photosystem II was inhibited, and the activity could be restored by adding electron donor of photosystem II (Shimazaki and Sugahara, 1979). However, Shimazaki and Sugahara(1980) have reported that DPC could not recover the rate of DCIP photoreduction inhibited by SO_2.

Silvius *et al.*(1975) showed that HSO_3^- much inhibited to oxygen evolution than SO_3^{2-} and SO_4^{2-}. Ziegler(1972) has demonstrated that RuDP-carboxylase is inhibited by SO_3^{2-}, and it was shown earlier that SO_4^{2-} can also affect this enzyme. The interference to CO_2 fixation also helps to explain the SO_2 effects on oxygen evolution in entire chloroplasts.

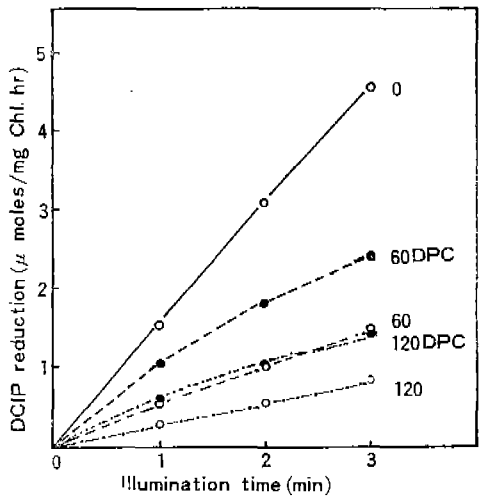

Fig. 15. Effect of DPC on DCIP photoreduction of barley leaves with 50 ppm SO_2 fumigation(0, 60, 120 indicate fumigation time, min., respectively).

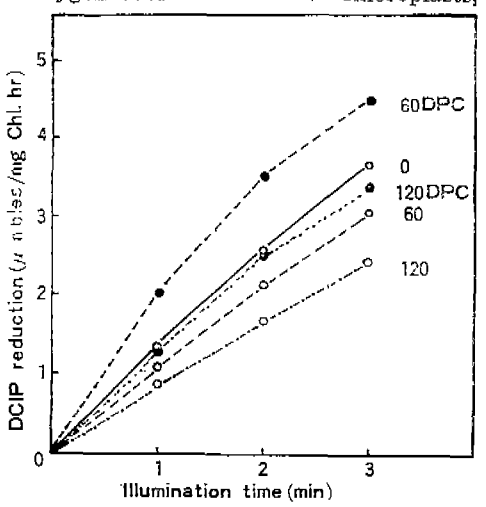

Fig. 16. Effect of DPC on DCIP photoreduction of corn leaves with 50 ppm SO_2 fumigation.

since a decrease in CO_2 fixation will indirectly affect oxygen evolution by PS Ⅱ.

In contrast to SO_4^{2-}, SO_3^{2-} is a strong ligand and bind to iron/heme-containing enzyme centers(Hällgren, 1978). The formation of metal complexes may, at least in part, help to SO_2 blockage of metalloenzyme-mediated processes in photosynthesis. Moreover, earlier investigation reported an increase in water-soluble "chloroplast iron" which was proportion to the duration of fumigation. The chloroplast iron is now known to represent ferredoxin and cytochromes in the photosynthetic electron transport chain. The entities which directly exert inhibitory action on photosystem II during SO_2 fumigation has carrying on.

It is concluded that corn is relatively resistant to SO_2 injury for pigments and protein content, catalase, peroxidase, polyphenoloxidase, and PS II activities, while barley is susceptible. It is supposed that corn has the biochemical adaptations to SO_2 toxicity.

摘 要

SO_2에 敏感한 보리와 比較的 抵抗性이 强한 옥수수에 SO_2를 處理하여 SO_2 毒性에 對한 反應 機作 및 生理的 影響을 比較 檢討하였다.

50 ppm의 SO_2를 2時間 處理하였을 때 잎 抽出液의 pH가 보리는 6.10에서 5.18로, 옥수수는 5.66에서 5.50으로 낮아졌으며, 酸에 對한 緩衝能은 옥수수 잎이 보리 잎에 比해 强한 것으로 나타났다. 옥수수 잎에서는 SO_2吸收에 依해서 생긴 SO_3^{2-}를 毒性이 적은 SO_4^{2-}로 酸化할 수 있는 能力이 높은 것으로 나타났다. 10 ppm의 SO_2를 處理함으로써 酵素의 活性度는 보리 잎에서는 catalase, 17% peroxidase 58% 및 polyphenoloxidase 88%로 감소하였으며, 옥수수 잎은 元來 catalase 活性이 아주 낮았으며, peroxidase는 136%, polyphenoloxidase는 128%로 增加하였다. 50 ppm의 SO_2를 2時間 處理하였을 때, 보리 잎의 carotenoid는 葉綠素보다 많이 減少하였으며 葉綠素 a/b는 4.1에서 3.6으로 減少하였다. 그러나, 옥수수 잎에서는 carotenoid가 葉綠素보다 많이 破壞되었으며 葉綠素 a/b는 1.9에서 1.8로 維持되었다. 可溶性 蛋白質 含量은 보리 잎에서는 59%, 옥수수 잎에서는 89%로 減少하였으며, 蛋白質 破壞率은 色素 破壞率보다 높았다. SO_2 處理에 依하여 色素 및 可溶性 蛋白質의 減少率은 보리 잎이 옥수수 잎에 比하여 훨씬 높은 것으로 나타났다. 50 ppm의 SO_2를 2時間 處理하여, 잎에서 抽出한 葉綠體의 DCIP 光還元率은 보리 18%, 옥수수 67%로 抑制되었으며, SO_2에 依하여 抑制된 DCIP의 光還元率은 人工電子供與體인 DPC를 添加함으로써 各各 32%, 92%로 回復되었다.

以上의 實驗結果로 옥수수는 보리에 比해서 生理的으로 SO_2毒性에 抵抗性이 强한 特性을 가지는 것으로 생각된다.

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