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Growth Responses of Lettuce Seeds Germinated in Aqueous Solutions of Sulfur Dioxide

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亞黃酸 수용액에서 發芽시킨 상치 種子의 生長反應

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ABSTRACT

Growth responses of lettuce seeds germinated in aqueous solutions of SO_2 were investigated for mode of action studies of this gas. The concentration and *p*H of SO_2 solution greatly affected the growth of germinating seeds. Root growth was more sensitive to SO_2 than hypocotyl growth. The sensitivity of total seedling growth lay between root and hypocotyl growth. Growth inhibition of germinating seeds appeared more serious when SO_2 was added during the early stage of germination.

INTRODUCTION

The injurious effect of dissolved sulfur dioxide (SO_2) on plant tissues is well recognized (Thomas *et al.*, 1950), but the toxic mechanism of this pollutant is still ambiguous. Phytotoxicity of SO_2 is partly attributable to its bleaching action (Lee *et al.*, 1975; Rao and LeBlanc, 1965; Puckett *et al.*, 1973). Even though its major injury to vegetation is leaf destruction (Thomas, 1961), an investigation on the growth response of developing plant systems toward SO_2 is of profound significance to elucidate the mode of action of this gas.

In germinating seeds, the imbibition of water is the primary reaction and metabolic activities are initiated during this process (Ching, 1972; Varner, 1965). Thus, germinating seeds afford a convenient system for studying the effects of dissolved SO_2 on plant growth or metabolism. The present experiments were undertaken to examine the growth pattern of germinating lettuce seeds as influenced by SO_2.

MATERIALS AND METHODS

Growth measurement was taken from the seedling height, root or hypocotyl length, and fresh or dry weight of entire seedlings. Three commercial varieties of leaf lettuce (*Lactuca sativa* L.) were employed throughout the experiments. Seeds of 'Grand Rapid' cultivar were purchased from Ferry-Morse Seed Company (U.S.A.) while those of 'Green Curled' and 'Red Curled', Korean native cultivars, were locally produced by Heung-Nong Seed Company.

Sodium sulfite was used as an SO_2 source (Lee *et al.*, 1975). SO_2 concentrations were based on stoichiometric values. Concentrations of SO_2 included 0, 200, 400, 600, 800, and 1,000 μg/ml at *p*H 7, and initial *p*Hs were 3, 5, 7, 9, and 11 at an SO_2 concentration of 600 μg/ml.

Twenty five or fifty seeds were germinated on a sheet of Whatman No. 1 filter paper in petri dishes containing 5 ml of the required solution. For the SO_2 treatment, seeds were germinated in water or SO_2 solution for one to three days, and in Hoagland solution for the remaining germination period. For a time-course study, seeds were directly germinated in a Hoagland solution and fresh nutrient solution was added as needed. Germination was accomplished at 28—30°C under 500 ft-c fluorescent light.

RESULTS AND DISCUSSION

Growth is commonly thought of in terms of increase of size, often to be measured by length or weight, and their changes may be plotted against time. The time course of growth in length of germinating lettuce seeds was measured at daily intervals. Growth curves of the entire seedling, root and hypocotyl are depicted in Fig. 1.

Seedling height and root length produced typically sigmoid growth curves, while hypocotyl length showed a very slight increase during a week. It is well recognized that growth curves can be divided into three distinct regions (Priestley and Pearsall, 1922). Under the present experimental condition, growth in length was an exponential function of time in the initial two days, and the rate of growth was directly proportional to time in the next three days. The growth rate fell off in the final two days.

Since the appropriate magnitude of growth was obtained in four days, the effect of SO_2 concentration on growth in length was examined with 4-day old seedlings. Fig. 2A shows that growth in length is gradually reduced with increasing concentrations of SO_2 and then greatly retarded at 600 μg/ml or higher concentrations of SO_2.

Seeds were almost completely germinated in the negative control and two lowest concentrations of SO_2. However, the germination was inhibited 7% at 600 μg/ml, 36% at 800 μg/ml and 53% at 1,000 μg/ml of SO_2. Approximately 30—40% of total seeds had no measurable hypocotyls even though they were germinated. An early worker (Barton, 1940) reported that SO_2 gas reduced the germination of soaked seeds.

Growth of seedling height, root, and hypocotyl length was converted into the percent of control (Fig. 2B). Root growth was more sensitive to SO_2 than hypocotyl growth. The sensitivity of total

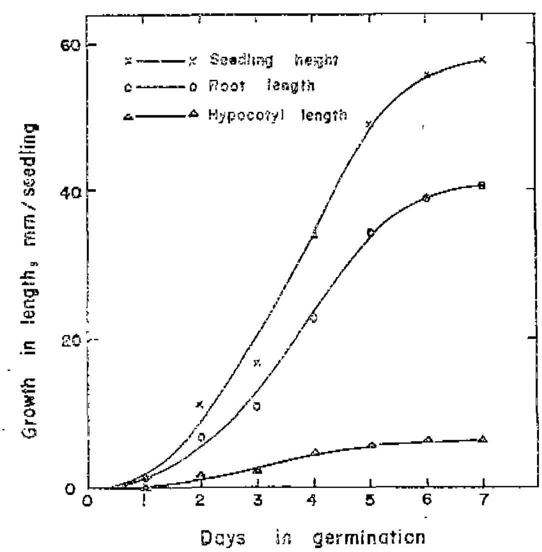

Fig. 1. Time course of growth in length of germinating lettuce seeds.

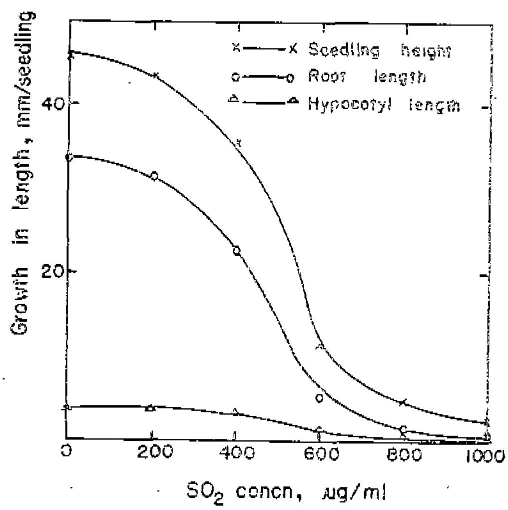

Fig. 2A. Absolute growth in length of lettuce seeds in 4 days germinated in various concentrations of SO_2 solution. Initial pH of SO_2 solution was adjusted to 7.

seedling height lay between root and hypocotyl growth.

As the SO_2 concentration of 600 μg/ml severely inhibited growth in length, this was chosen for further studying the effect of SO_2 on the growth in length. Growth of seedling height, root and hypocotyl length was measured at 2-day intervals. As shown in Fig. 3, growth of SO_2-treated seedlings was increasingly suppressed as time elapsed.

The *p*H of the SO_2 solution at a definite concentration appeared to affect greatly the growth of seedling height, root and hypocotyl length(Fig. 4). Seedling or root growth comparable to the control was shown only at *p*H 9. Seedling or root growth was somewhat suppressed at *p*H 11, but greatly suppressed at *p*H 7. None of seeds were germinated in the acidic solution. No discernible difference of hypocotyl growth was shown between *p*H 9 and 11.

It was shown in Fig. 5 that growth inhibition of germinating seeds was more serious when SO_2 was added during the early stage of germination. Seedling or root growth was somewhat less susceptible when SO_2 was added at the emergence of the radicle from the seed coat than when added from the beginning of germination, and far more susceptible when SO_2 was added one day after radicle protrusion. The susceptibility of seedling or root to SO_2 was gradually decreased as seedlings became larger at the time of SO_2 addition. Hypocotyl lengthening was somewhat more inhibited when SO_2 was added prior to or at the emergence of the radicle than thereafter.

Growth pattern as influenced by SO_2 was also investigated in terms of fresh or dry weight. According to Fig. 6, the time course of fresh weight also showed a typically sigmoid growth curve, however, the dry weight showed a very slight increase up to 7 days.

When seeds were germinated in various concentrations of SO_2 solution, fresh weight of 4-day old seedlings was slightly increased at 200 μg/ml, compared to the control, and gradually decreased with increasing concentrations of SO_2(Fig. 7).

Low concentrations of SO_2 could be beneficial to plant growth in sulfur-deficient soil or nutrient (Katz, 1949). As seeds were germinated in water or aqueous solution of SO_2 lacking all the essential elements, a slight increase of fresh weight in the low concentration of SO_2 might be attributed to

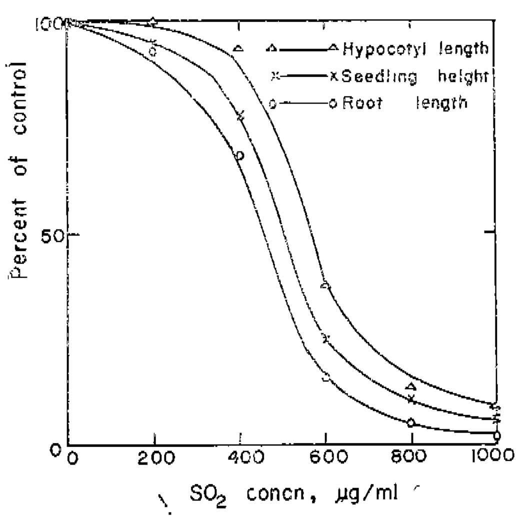

Fig. 2B. Relative growth in length of lettuce seeds germinated in various concentrations of SO_2 solution.

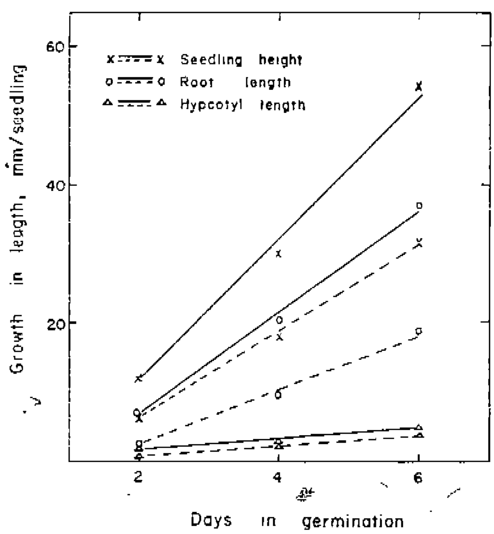

Fig. 3. Growth in length of lettuce seeds germinated in water (——) and 600 μg/ml of SO_2 solution (- - -) as measured at 2-day intervals.

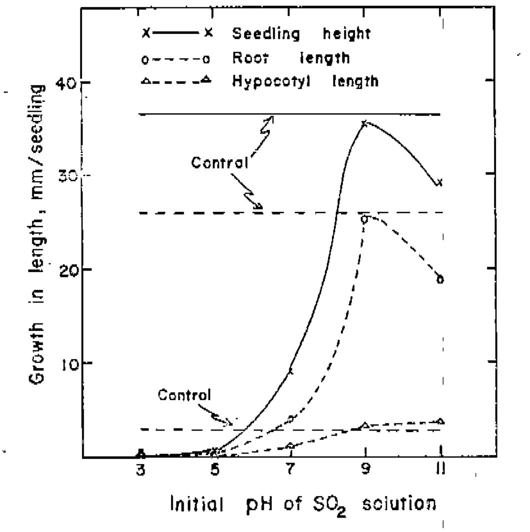

Fig. 4. Growth in length of lettuce seeds germinated in various initial *p*Hs of SO_2 solution. Stoichiometric concentration of SO_2 solution was 600 μg/ml.

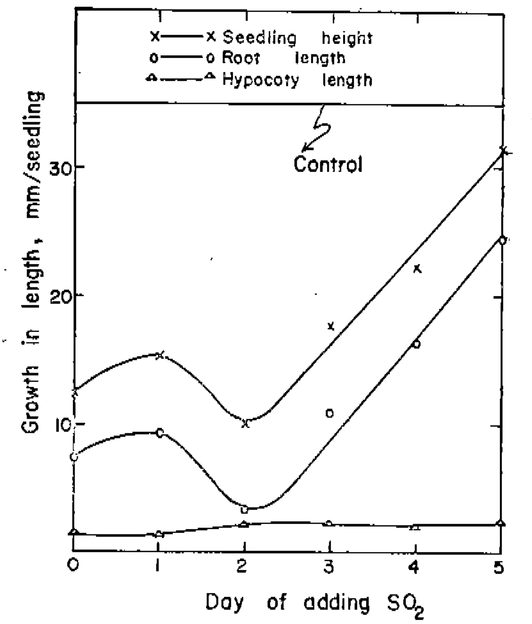

Fig. 5. Growth in length of germinating lettuce seeds on addition of SO_2 at different days. Stoichiometric SO_2 concentration and initial *p*H was 600 μg/ml and 7, respectively.

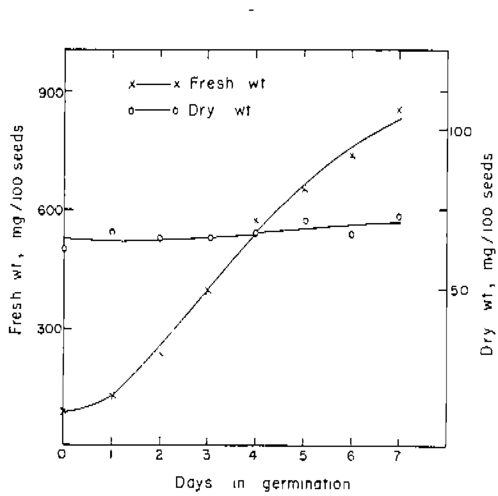

Fig. 6. Time course of growth in weight of germinating lettuce seeds.

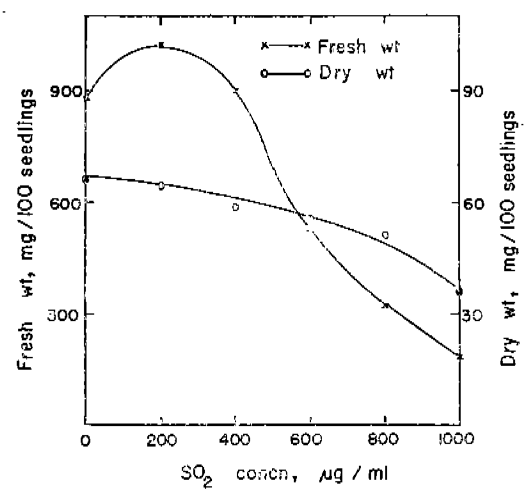

Fig. 7. Growth in weight of lettuce seeds in 4 days germinated in various concentrations of SO_2 solution.

the availalble sufur supply for germination.

As shown in Fig. 8, 600 μg/ml of SO_2 inhibited the increase of fresh or dry weight of seedlings measured at 2-day intervals. The degree of inhibition became greater as time elapsed.

None of the seeds were germinated in initially acidic SO_2 solutions. Fresh weight of 4-day old seedlings was considerably decreased when seeds were germinated in initially neutral solutions, but enhanced in initially alkaline solutions. Dry weight represented a somewhat different response. Dry weight was decreased in initially acidic or neutral solutions and slightly increased in initially alkaline solutions (Fig. 9).

According to Fig. 10, fresh or dry weight of 4-day old seedlings was considerably decreased when seeds were germinated in SO_2 solution from the beginning or treated by SO_2 solution a day after imbibition, comparing with the control. However, it was gradually increased, if not reaching the control, when SO_2 was added afterwards.

All the above results clearly indicated that the effect of SO_2 concentration or *p*H on the growth of germinated seeds was well coincided with that leading to the discoloration of leaf disks (Lee *et al.*, 1975). This fact may suggest that the mechanism inhibiting the growth of germinated seeds is essentially the same as the mechanism of leaf discoloration.

In the previous report(Lee *et al.*, 1975), discoloration was attributed to nascent hydrogen produced as sulfurous acid is oxidized to sulfuric acid by combining with water. The hydrogen atom was presumed to convert chlorophyll into phaeophytin in leaf disks replacing the magnesium atom under acidic conditions (Lee *et al.*, 1975; Rao and LeBlanc, 1965). It might also be possible that the hydrogen atom exerts toxic action by reducing "hydrogen acceptors" such as coenzymes in germinating seeds.

However, a recent study on the chemical changes of SO_2 solution *in vitro* revealed that the release of hydrogen was decreased as initial *p*H of the SO_2 solution became lower (Lee and Lee, 1975). The result seems rather contradictory, as discoloration was more serious and germination was more severely inhibited at low *p*H values.

Unionized sulfurous acid molecules might exert toxic action on a germinating system in a specific manner. Even though sulfurous acid itself does not exist, two types of salts containing bisulfite or sulfite ions are well recognized and there appear to be several molecular species in bisulfite solutions (Cotton and Wilkinson, 1966). Therefore, it is also feasible that one of tautomers of bisulfite ions prevalent at low *p*H plays an important role in the toxic mechanism. Gilbert (1968) previously suggested that unionized sulfurous acid molecules

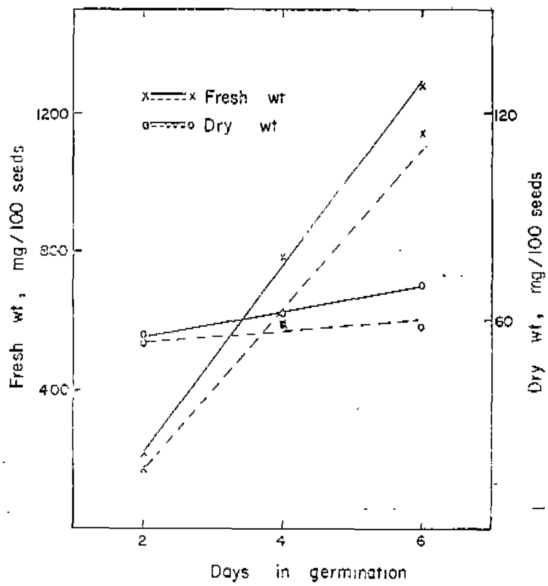

Fig. 8. Growth in weight of lettuce seeds germinated in water (——) and 600 μg/ml of SO_2 solution (- - -) as measured at 2-day intervals.

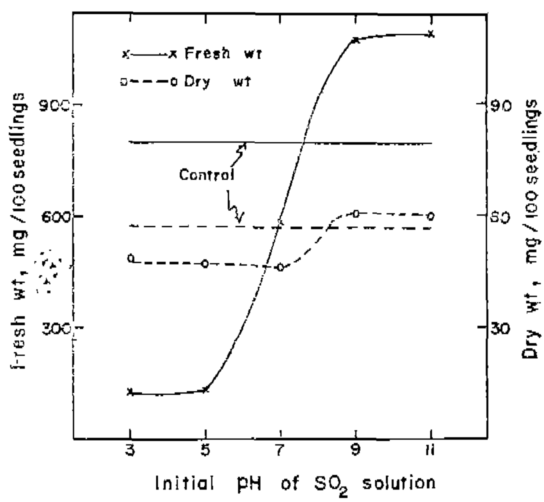

Fig. 9. Growth in weight of lettuce seeds germinated in various initial *p*Hs of SO_2 solution. Stoichiometric SO_2 concentration was 600 μg/ml.

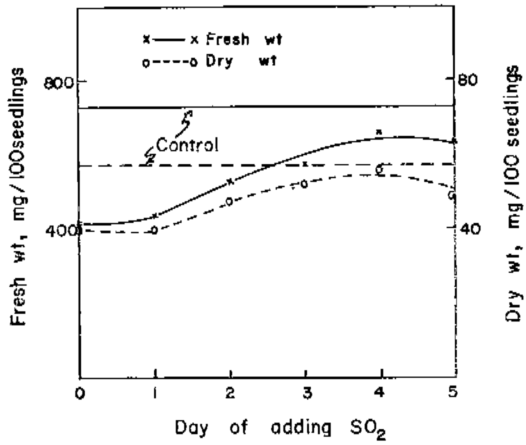

Fig. 10. Growth in weight of germinating lettuce seeds on addition of SO_2 at different days. Stoichiometric SO_2 concentration and initial *p*H was 600 μg/ml and 7 respectively.

and bisulfite ions were toxic forms in aqueous solutions.

Kamagawa and Fukui (1973) showed that bisulfite ions inhibited α-glucan phosphorylase by competing with phosphate at the phosphate binding site of the enzyme. Tanidazawa *et al.* (1972) suggested that SO_2 reduced ribulose-1, 5-diphosphate carboxylase activity, as sulfite blocked the sulfhydryl group of the enzyme protein. Mukerji and Yang (1974) found that phosphoenolpyruvate carboxylase, partially purified from spinach leaves, was inhibited by sulfite ion.

Even though the biochemical effect of SO_2 at the enzymic level is somewhat known, the specific mechanism of phytotoxicity is still uncertain. Apparently a further mode of action study matched to the growth response of germinating seeds would be helpful to elucidate the toxic mechanism of SO_2.

摘 要

SO_2 被害機作 연구의 일환으로 發芽하는 상치종자의 生長反應을 조사하였다. 發芽종자의 生長은 SO_2 수용액의 농도 및 *p*H에 의하여 크게 영향을 받았다. 뿌리 生長은 胚軸生長보다 SO_2에 더욱 敏感하였으며 총길이 生長의 敏感度는 뿌리 및 胚軸生長의 중간에 위치하였다. 發芽종자의 生長억제는 發芽초기에 SO_2를 처리하였을 때 더욱 심한 경향을 보였다.

REFERENCES

Barton, L. V. 1940. Toxicity of ammonia, chlorine, hydrogen cyanide, hydrogen sulphide, and sulphur dioxide gases. *Contrib. Boyce Thompson Inst.* 11 : 357—362.

Ching, T. M. 1972. Metabolism of germinating seeds, p. 103—218. In: T. T. Kozlowski [ed.] Seed Biology, Academic Press, New York.

Cotton, F. A. and G. Wilkinson. 1966. Advanced Inorganic Chemistry, Interscience Publishers, New York. 1136 p.

Gilbert, O. L. 1968. Bryophytes as indicators of air pollution in the tyne valley. *New Phytol.* 67 : 15—30.

Kamagawa, A. and T. Fukui. 1973. Inhibition of L-glucan phosphorylase by bisulfite competition at the phosphate binding site. *Biochim. Biophys. Acta.* 302 : 158—166.

Katz, M. 1949. Sulur dioxide in the atmosphere and its relation to plant. life. *Ind. Eng. Chem.* 41 : 2450—2465.

Lee, M. S. and S. R. Lee. 1975. Chemical changes of dissolved sulfur dioxide *in vitro*. *J. Korean. Agr. Chem. Soc.* 18 : (In Press).

Lee, M. S., S. W. Park, and S. R. Lee. 1975. Discoloration pattern of lettuce leaf disks as influenced by sulfur dioxide. *Korean J. Botany* 18 : 1—10.

Mukerji, S. K. and S. F. Yang. 1974. Phosphoenolpyruvate carboxylase from spinach leaf tissue. Inhibition by sulfite ion. *Plant Physiol.* 53 : 829—834.

Priestley, J. H. and W. H. Pearsall. 1922. Growth studies. II. An interpretation of some growth curves. *Ann. Botany* 36 : 239—249.

Puckett, K. J., E. Nieboer, W. P. Flora, and D. H. S. Ricdson. 1973. Sulfur dioxide: its effect on photosynthetic ^{14}C fixation in lichens and suggested mechanisms of phytotoxicity. *New Phytol.* 72 : 141—154.

Rao, D. N. and B. F. LeBlanc. 1965. Effects of sulfur dioxide on the lichen alga, with special reference to chlorophyll. *Bryologist* 69 : 69—75.

Tanidazawa, M., K. Furubashi, and K. Tanaka. 1972. Experimental studies on the injury of plants by SO_2 gas (Part 6). *J. Japan Soc. Air Pollution* 7 : 229.

Thomas, M. D. 1961. Effects of air pollution on plants, In Air Pollution, World Health Organ., Monogr. Ser. No. 46, p. 233—278, Columbia Univ. Press, New York.

————, R. H. Hendricks, and G. R. Hill. 1950. Sulfur metabolism of plants. Effects of sulfur dioxide on vegetation. *Ind. Eng. Chem.* 42 : 2231—2235.

Varner, J. E. 1965. Seed development and germination, p. 763—792. In: J. Bonner and J. E. Varner [ed.] Plant Biochemistry, Academic Press, New York.

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