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EFFECT OF ETHYLENE ON SPROUT GROWTH AND ENDOGENOUS GROWTH SUBSTANCES OF POTATO PLANTS

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Introduction

Regulation of potato sprouting and its further development through the exogenous application of several chemicals have been receiving considerable attention. Since ELMER⁹⁾ in his classical work observed shortening and swelling of sprouts in growing potatoes exposed to ethylene produced by ripening apples. Many studies on the physiological role of ethylene in the process of potato sprouting were extensively performed from diverse angles. Several lines of evidence, including stimulation or inhibition of the sprouting^{4,14,15)}, increase in respiration and sugar content^{5,13)}, and altering some enzymes activities^{7,16)} have been accumulated. In recent years, a work of RYLSKI *et al.*¹⁵⁾ has advanced the discussion on the dual effect of ethylene on the potato sprouting.

More detail study pursued by Catchpole and HILLMAN⁹⁾ has substantiated an obstruction of potato sprout development elicited by ethylene treatment from morphological and anatomical points of view. Although ethylene has been manifested to produce a pronounced effect on metabolism of endogenous growth substances in many plants⁹⁾, a limited amount of information is available concerning changes in levels of growth substances in the sprouting potato tubers caused by ethylene. In this experiment described below, levels of individual endogenous growth substances in potato tissues were measured in an attempt to evaluate their possible involvement in the sprouting of ethylene-treated potato tubers.

Materials and Methods

Plant materials: Potato tubers (*Solanum tuberosum* L. cv. Irish Cobbler) supplied by the Experimental Farm of Hokkaido University were used

for materials throughout the present experiment. The tubers averaging 300 g in weight were sorted and washed free of soil, and then they had been stored in a cellar until the end of dormant period. Requiring for experiments, they were surface-sterilized with 0.5% sodium hypochlorite solution followed to wash thoroughly with running tap water and blotted dry.

Ethylene treatment of tubers: Immediately after removing from storage, lots of forty tubers were placed right-side up in a gas-tight plastic cabinet (45×30×14 cm) held in a dark room at 20°C. A constant stream of purified air with or without ethylene was led over the tubers in the cabinet at a flow rate of 100 ml per minute. The air supply system conducting from outdoor was essentially the same as described in the previous paper¹²⁾. A desired concentration of ethylene at 2 ppm was prepared by mixing 100 ppm of ethylene with purified air and the rate of mixing was precisely accomplished with the aid of micro-pump. The ethylene concentration in the air stream was periodically verified by flame ionization gas chromatography.

Growth analysis: Concerning sprout growth of tubers, their fresh and dry weights, rates of sprouting and rooting, and length and width of sprout were used as parameters for assessing potentiation of sprouting and all values were on an average of 15 to 20 tubers in three replicates per treatment.

Histological observation: After receiving the treatments, sprouts excised from apical part of the tubers were immediately fixed in FAA (formalin-acetic acid-alcohol fixative), processed for microtomy in the usual way⁸⁾ and sectioned at 10 microns. The preparations were stained with safranin-fast green or PAS⁹⁾ (periodic acid-Schiff's reagent).

Extraction, fractionation and bioassay of auxin and inhibitor: Cylindrical tissue plugs weighing 10 g were prepared from different part of tubers, *i. e.* apical tissue with buds, cortical tissue including periderm without buds, and central pith tissue. Each of them was chopped into slices 1 mm thick and immersed to extract with three changes of 300 ml diethylether (peroxide free) for 48 hr at 3°C with occasional shaking. After filtering the combined extracts, the remained tissues were washed with 300 ml ether. The extract was evaporated to dryness at 30°C *in vacuo*. The resulting residue was redissolved in 10 ml of 1 M sodium bicarbonate solution and extracted with three equal volumes of ether. After discarding the ether phase, the remaining aqueous solution was acidified to pH 2.7 by the addition of 1 N HCl and reextracted four times with equal volumes of ether. The bulked ether phase was dried over anhydrous sodium sulphate at 3°C over night, filtered and reduced to dryness. The ensuing residue was taken

up in a small volume of 80% ethanol, streaked on to Toyo No. 50 chromatography paper and then separated with iso-propanol: NH_4OH : water (10:1:1, v/v). Dried chromatograms were divided into 10 Rf strips and those below Rf 0.4 were used for auxin assay and the remained ones were for inhibitor assay.

Biological activities of these substances were assayed by *Avena* coleoptile straight growth test devised by NITSH and NITSH¹⁰. Only for determination of inhibitor activity, assay medium was fortified with indole-3-acetic acid (IAA) at 0.05 mg/l in order to make the activity more distinguishable.

Results

Effect of ethylene on emergence and development of potato sprout :

Whether bud of potato tuber starts to grow or not was distinguished by an appearance of white tiny sprout as an earliest sign of emergence.

Changes with time in sprouting rate of potato buds are shown in Fig. 1 and 2, which indicate that onset of the sprouting was a few days earlier in apical buds than lateral ones. The fact is considered to take place due to apical dominance. Following the ethylene treatment, however, not only retardation of sprouting in the apical buds but enhancement of the lateral ones occurred. From these observation, it is clear that the ethylene treatment would elicit a pronounced effect on abolishing the apical dominance. So far as concerning the present experiment, a commencement of sprouting

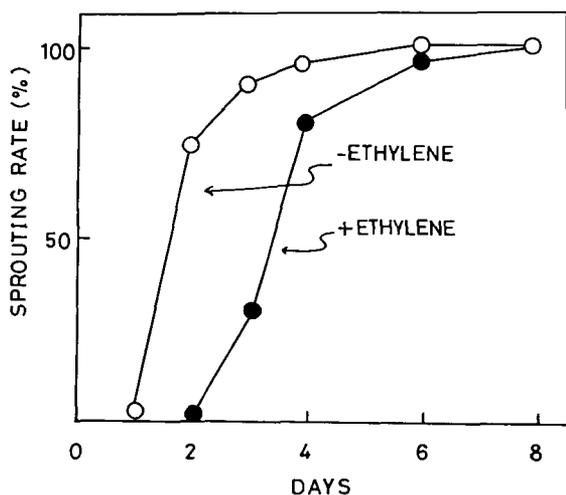


Fig. 1. Sprouting rate of apical bud of potato tubers continuously treated with ethylene at 2 ppm.

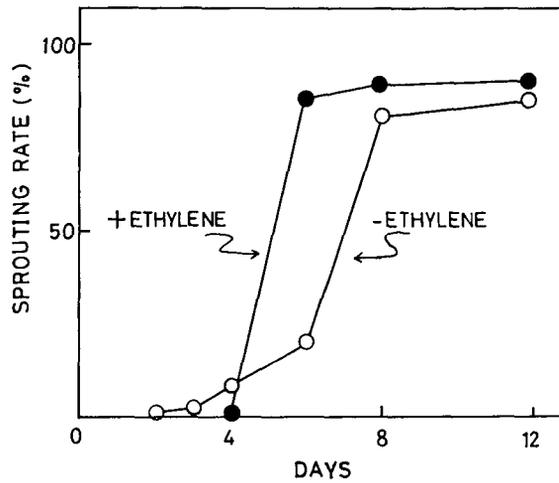


Fig. 2. Sprouting rate of lateral buds of potato tubers continuously treated with ethylene at 2 ppm.

of either apical buds or lateral ones was unable to be enduring arrested, irrespective of whether they were received ethylene treatment or not. On the other hands, outgrowth of adventitious roots originated from flank of the sprouting buds was observed soon after the sprouting. The number of the buds with the vigorous elongating roots became to increase with time,

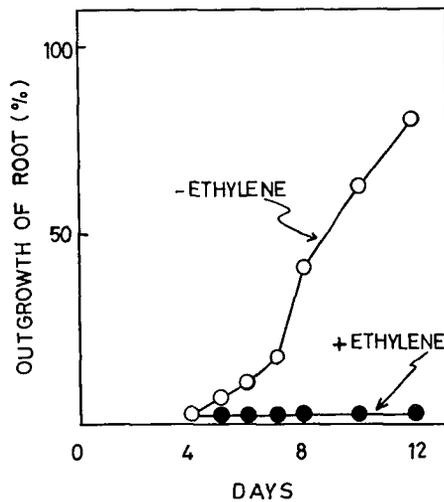


Fig. 3. Changes in outgrowth of adventitious root originated from flank of the sprouting buds of potato tubers treated with ethylene at 2 ppm.

and the numbers were represented on a percentage basis per tuber as rooting rate (Fig. 3). It is evident that the root elongation was completely suppressed with the ethylene treatment.

In an additional evidence showing in Fig. 4 photographically, there were found copious elongated roots along surface of the tuber while no visible root elongation was detected in all side of the sprouts of the ethylene-treated tubers even at 4 weeks after incubation. The effect is also substantiated by the results of histological observation present in Fig. 5. Transverse section of either the ethylene-treated or non-treated sprouts showed occurrence of root primordia regenerated endogenously from endodermis tissue of the sprouts. At the end of the extended ethylene treatment for four weeks, however, physical appearance and properties of the sprouts were affected by the gas. Swelling and decrease in longitudinal growth of the sprouts resulted from the continuous ethylene treatment as showing in Fig. 4 and

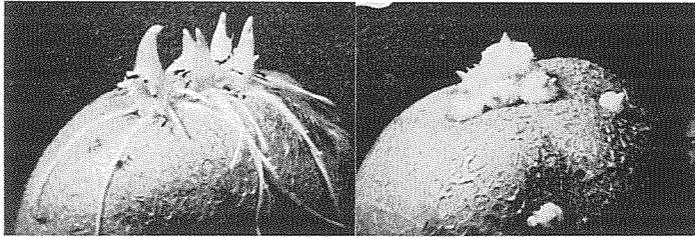


Fig. 4. Appearance of sprouts emerged from potato tuber treated with ethylene (right) and control (left). The figures were taken at the end of 4th week incubation.

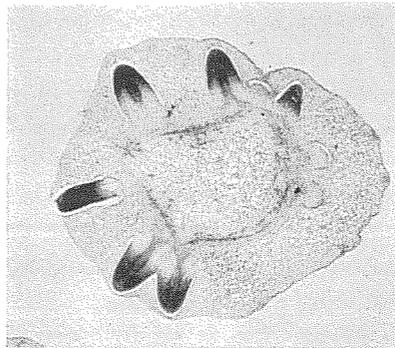


Fig. 5. Transverse section of basal region of swollen sprout emerged from ethylene treated potato tuber. The section was stained with safranin-fast green. Note that there are many root primordia originated from endodermis of both control and ethylene treated sprouts.

TABLE 1. Effect of ethylene treatment on sprout growth of potato tuber

Treatment	Length (mm)	Width (mm)	Fresh weight (mg/sprout)	Dry weight (mg/sprout)
Control	11.8	3.6	91.7	10.6
Ethylene	7.7	5.9	163.8	24.5

Forty potato tubers were exposed continuously streaming air with or with or without ethylene (2 ppm) at 20°C for 4 weeks in darkness.

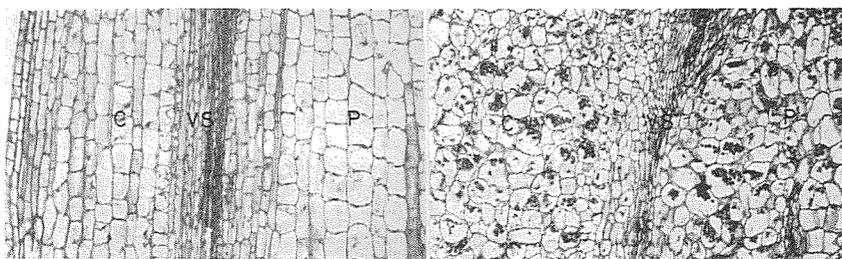


Fig. 6. Longitudinal sections of sprouts of ethylene treated tubers (right) and control (left). Note that much accumulation of starch grains was detected in the cells of ethylene treated sprouts. C=cortex, VS=vascular bundle, P=pith,

Table 1, and cortical and pith cell lengths were decreased in swollen region of the thicked sprouts (Fig. 6). In addition, of interesting is the fact that a much higher amount of starch grains was accumulated in the cells of the treated sprouts in compared with the control ones (Fig. 6). Comparing the fresh and dry weights between the sprouts with and without ethylene, it was resulted in appreciable increases in not only the fresh weight but the dry one due to the ethylene treatment, being accompanied with starch accumulation in their cells (Fig. 6).

Effect of ethylene on changes in growth substances of the sprouts:

A gross difference in the outward appearance of the sprouts treated with ethylene suggests to undergo a shift in metabolism of endogenous growth substances and this possibility was investigated. From an inspection of the data illustrating in Fig. 7 and 8, it is readily seen that the ethylene treatment caused a drastic decline in auxin activity in the apical part of the tubers and only a slight changes in level of growth inhibitor, if any. Somewhat less but likewise decline in auxin activity was also found in the other part of the tubers owing to the treatment, except central pith tissues in

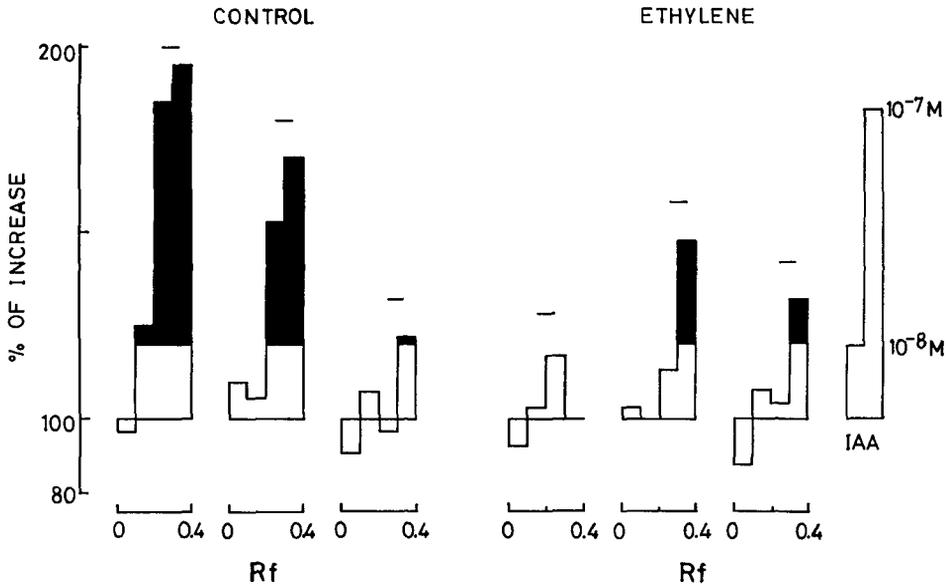


Fig. 7. Histograms of endogenous auxin activities extracted from apical buds (left), cortical tissues (middle) and pith tissues (right) of potato tubers treated with and without ethylene. Acidic ether fractions of ethanol extracts prepared from each tissue were chromatographed with iso-propanol: 28% water (10:1:1, v/v) and assayed with *Avena* coleoptile test. Horizontal bars indicate migrating region of marker IAA and vertical open bars at right side indicate standard activity of IAA at 10^{-7} and 10^{-8} M.

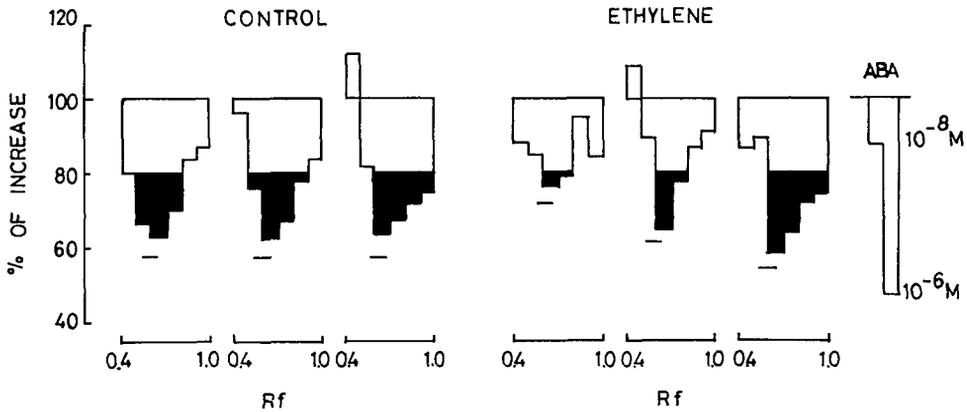


Fig. 8. Histograms of inhibitors extracted from apical bud (left), cortical tissue (middle) and pith tissue (right) of potato tubers treated with and without ethylene. The inhibitory activities were assayed as the same manner of Fig. 7. The assay medium was added with IAA 0.05 mg/l. Horizontal bars indicate migrating zone of ABA (Rf 0.7). Vertical open bars at right side indicate standard activity of ABA at 10^{-6} and 10^{-8} M.

which a slight increase of the activity resulted. The distribution of the growth substances on chromatograms clearly indicates that a major peak of auxin activity was considered to be IAA and a potent inhibitor migrated to Rf 0.6–0.8 is quite resemble to the Rf of abscisic acid. The data lend preliminary support to a hypothesis that the occurrence of morphological disorder in the ethylene-treated sprouts would presumably be a reflection of the decrease in the auxin level other than the inhibitor.

Discussion

In the present study it has confirmed that ethylene exerted a pronounced effect on development of abnormal sprouts known as “knobby sprouts” and those emerged simultaneously from almost all of the eyes of tubers. From the available evidences⁶⁾, ethylene apparently led to retard growth of apical bud with shortened roots. In agreement with an earlier finding⁶⁾, an accumulation of starch grains in the cells of sprouts incited by ethylene was also worth notice. The reverse situation has been reported for the starch accumulation by CATCHPOLE and HILLMAN²⁾ that ethylene caused an abnormal swelling of stolon devoid of any appreciable starch accumulation. Although the reason for the discrepancy is not clear, it is possible to arise out of the fact that the sprout unlike stolon has much more amount of carbohydrates supplied directly from intact tubers which is rich in available source of starch. However, taking relatively higher concentration of ethylene used this experiment into consideration, it is difficult to draw an exclusive conclusion due to these complicating factors. It has generally been known an inhibition of root growth caused by ethylene treatment¹⁾. In this context, MINGO-CASTEL *et al.*¹⁰⁾ have also worked on effect of ethylene on potato root development and stated inhibitions of both root formation and its elongation. As showing in Fig. 5, however, we also confirmed that due to the ethylene treatment visible elongation of root-germ formed on the potato sprouts was strongly inhibited whereas there was little or no inhibition of the root-germ formation. HUGHES *et al.*⁶⁾ have already pointed out the fact that the inhibition of root elongation could be reversed by removal from ethylene, which seems to imply that normal root formation is always preceded even if subjecting to ethylene, and the present data lend to support this assumption.

The progenies of the ethylene-treated tubers had a much extractable auxin content in the tuber tissues surrounding apical bud. The fact seems possible to be closely associated with the developmental aberration of the sprouts. Lowering of auxin levels in plant tissues due to either destruction

of auxin or inhibiting its biogenesis caused by ethylene treatment has been suggested and extractability of auxin from plant tissues was also possible to alter by the treatment. In fact, an evidence⁹ that IAA levels are diminished in pea epicotyls treated with ethylene is additionally support for this assumption, and this assertion is much strengthened by the result presented in Fig. 7. However, since there is as yet not much evidence, it is not possible to offer any comment regarding the metabolic fate of endogenous auxin. Biochemical attempts are now under progress in order to elucidate the auxin metabolism in the ethylene-treated potato tubers.

Summary

The present investigation is concerned with effect of ethylene treatment on potato sprouting. Ethylene elicited to lose apical dominance of potato tuber leading to multi-sprouting, and shortened and thickened symptoms were also characteristics of the sprouts. Ethylene did not prevent spontaneous root formation but inhibited its further development. A lowering of auxin content in the tuber tissues surrounding the apical buds occurred by the ethylene treatment. Therefore it can be deduced that shortage of auxin in the apical bud may participate in inducing abnormal sprouting of tubers.

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