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Use of Plant Leaf in Biosensing for Some Odour Compounds

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Abstract—The sensing of odour compounds in gas phase is an attractive target in recent sensor technology. Based on the finding that a plant leaf can respond to various gas molecules by changing its potential, biosensing system using a plant leaf has been investigated for the detection of odour compounds. A leaf of some plant species responded to odour compounds directly by changing its potential 5~10 mV. That the leaf was actually sensing an odour was much more remarkably detected from the difference between the response profile to pure CO_2 gas and that to CO_2 gas containing odour compounds. Then the quantitative study (ppb level) is now being performed on the response of a tobacco leaf to benzyl acetate; a component of jasmine-like odours. The concept of biosensing and its significance are also described from the viewpoint of sensor technology.

1. Introduction

Since the development of an enzyme sensor in 1960s, quite a few works have been performed for the development of biosensors. Biomaterials used in biosensor systems include from single enzyme or antibody to a more complicated materials such as organelle, microorganism, and tissue.[1] They showed remarkable performance by responding to a specific chemical compounds. However, most of them were too unstable to be used for practical purposes. Until now, much efforts have been focused on how to improve their stability. However satisfactory methods have not yet been developed.

On the other hand, much attention has recently been paid to the use of a living thing such as animal or plant. A living thing seems much more attractive than isolated biomaterials from the viewpoints of stability and potentiality of excellent biofunctions. In order to construct a chemical sensor system using a living thing, it is necessary, first of all, to sense how the living thing is feeling to respective chemicals. Such a concept of sensing a living thing is called biosensing.

Our interest exists in biosensing systems using plant, because plant seems to have many advantages from the practical viewpoint. As described below plant might have an excellent sensing function for gaseous chemicals including volatile components of essential oils which are generally called odour compounds. Thus our efforts have been concentrated on the development of biosensing system for the detection of odour compounds.

From the viewpoint of chemical sensing

technology, the detection of odour compounds is a most attractive target. In this line, many research works have been performed with chromatographic instruments,[2,3] rather simple devices or biomimetic systems,[4,5] and by theoretical model analyses.[6,7] However, no artificial system has succeeded in reaching the performance of animal olfaction. Thus odour sensing, odour evaluation, and odour preparation are, up to date, still conducted with use of animal olfaction.[8,9]

As is well known, plants are emitting or secreting various chemical compounds in the air or in the soil.[10~12] Recently it has been suggested that some of them are playing an important role in the chemical communication between plants. Based on these observations, the sensing function of plants to chemical compounds, especially to gaseous molecules, has been surveyed. As demonstrated by the electrochemical method, plant leaves responded to odour compounds in various manners.

This paper reviews the responsive properties of plant leaves to gaseous molecules including CO_2 gas and some odour compounds. The concept of biosensing is also described.

2. Response Of A Plant Leaf To CO_2 Gas

Plants take in CO_2 and O_2 gases through their stomata. Formerly Raschke used the term "CO_2 sensor" in relation to the feedback system for CO_2 uptake, though corresponding substances

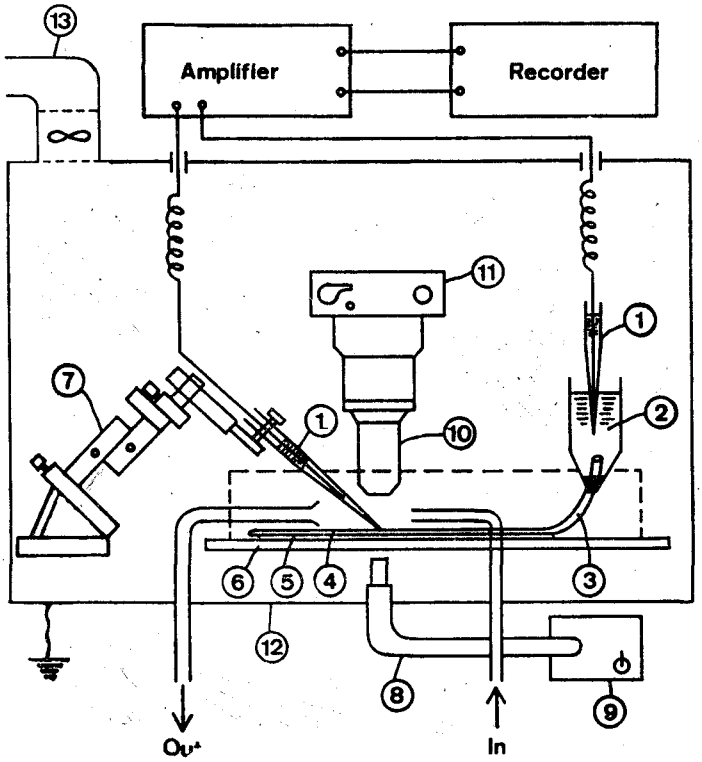

Fig. 1. Experimental set-up for the measurement of electrochemical responses of a leaf to gaseous chemicals. (1) Capillary electrode(electrode: Ag-AgCl, electrolyte:3 M KCl sat. AgCl). (2) bathing solution(KH_2PO_4-K_2HPO_4 ([K] : 0.3 M), NH_4Cl(0.1 M), pH 6.5). (3) stem. (4) leaf. (5) double-sided adhesive tape. (6) slide glass. (7) micromanipulator. (8) photoguide. (9) lamp box. (10) microscope. (11) camera. (12) shield box. (13) blower.

were not identified.[13] The movement of stomata of plant leaves has been thoroughly investigated in relation to CO_2 gas concentration, humidity, and light illumination conditions. Changes in stomata opening rate were observed according to the variation of these factors. The possible role of proton and potassium ion in the movement of stomata was also discussed. From this information, it was expected that a plant leaf might respond to CO_2 gas by changing the cellular potential of a leaf.

Initially a spider wort(*Commelina communis*) was studied. A sample leaf of *C. communis* was cut off at the stem before use and fixed on a glass plate with an adhesive tape. The back side of the leaf was faced upwards. The sample leaf was set under a microscope as illustrated in Fig. 1. The fixed leaf was observed with a microscope(×150). Light illumination was performed with a photo-guide from the lamp house. Light intensity was greater than 2.5×10^3 Wm^{-2} at the stage of the microscope.

The leaf potential was measured with a pair of capillary electrodes. The stem was dipped in a bathing solution composed of 0.1 M phosphate buffer solution(K_2HPO_4—KH_2PO_4, pH 6.5) and 0.1 M NH_4Cl, in which one electrode was immersed. The other electrode was inserted in a particular cell of the leaf with micromanipulator. The potential difference between the two electrode was detected with a high impedance amplifier and displayed on a recorder. The whole system was set on a vibration-proof bench in an electromagnetic shielded room. When 100% CO_2 gas was applied to the leaf under illumination, the leaf potential shifted immediately about 100 mV in the negative direction and then turned to the positive direction as shown in Fig. 2. Finally the leaf potential became about 50 mV higher than the initial level. When CO_2 gas supply ceased, the leaf potential moved immediately to the initial level. Similar responses were obtained repeatedly.

Response profiles were different depending upon the light condition and CO_2 gas concentration. Fig. 3 summarizes the results. For the case of light illumination and low CO_2 gas

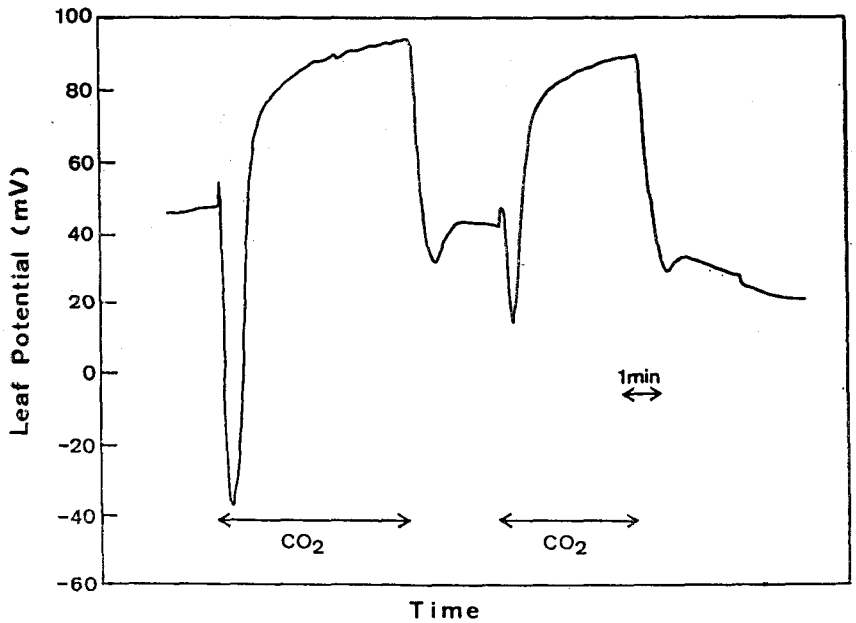

Fig. 2. Response of *C. communis* leaf to 100% CO_2 gas under illumination.

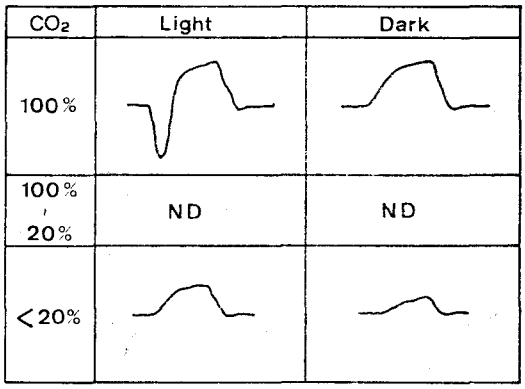

Fig. 3. Classification of response patterns depending upon the CO_2 gas concentration and light condition. ND means "measurement was not done"

concentration (less than 20%), the difference of leaf potentials at the initial level and the maximum or steady level (ΔV) was measured against CO_2 gas concentration. Fig. 4 shows the results obtained from one of the subsidiary vein cells. A positive correlation was obtained between ΔV and CO_2 concentration.[14] A similar correlation was also obtained under light shielding, though the magnitude of ΔV was reduced.

The stability of the leaf was checked by measuring the response to repeated exposures to 100% CO_2 gas under illumination. The measurement was performed once or twice a day. Otherwise, CO_2 gas supply was cut off and light was shielded. The measuring electrode was maintained inserted in the cell during the whole period. The leaf potential change gradually decreased and finally disappeared after 10 days.

Response to CO_2 gas was obtained from most of plant species assayed. Until now 25 species have been shown to respond. A correlationship between ΔV and the CO_2 gas concentration was also obtained from such species as *Ficus benjamina*, *Lactuca stolonifera*, *Gardenia jasminoides*, and *Nicotiana tabacum*.

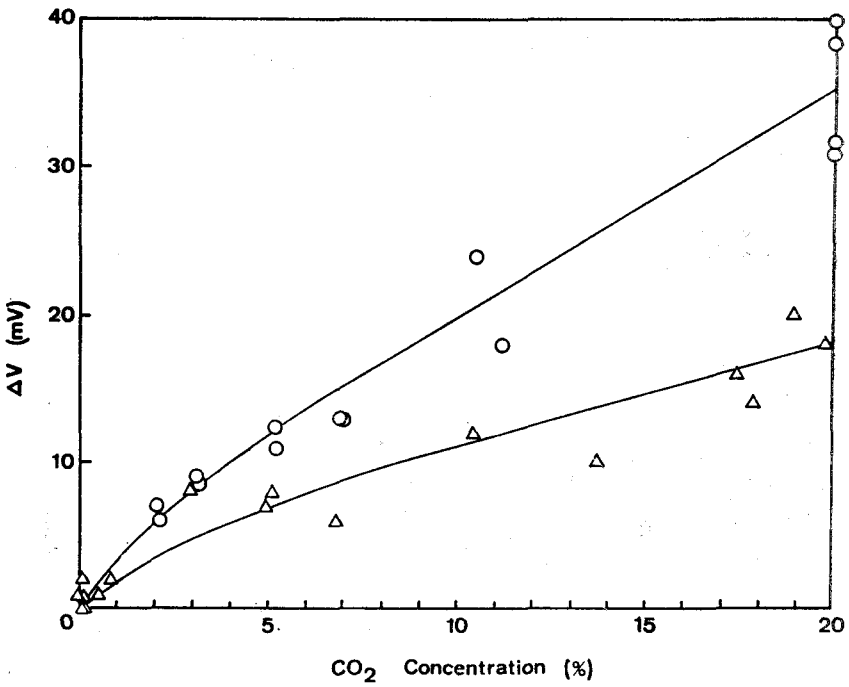

Fig. 4. Dependence of CO_2 gas concentration(less than 20%) on ΔV. (○): under illumination, (△): in the dark.

3. Energy Requirement In Response To CO_2 Gas

Formerly, it was demostrated that CO_2 gas (10～50%) caused the hyperpolarization of the hypocotyl of *Vigna sesquipedalis*.[15] That hyperpolarization was ascribed to the activity increase of electrogenic ion pump. Thus similar phenomena observed for leaves are suspected to involve the same mechanism.

In order to check weather the response of tobacco leaf to CO_2 gas requires energy, we developed a leak-tight reaction vessle for the pontential measurement of a whole leaf without light nor oxygen as depicted in Fig. 5. A sample leaf was attached on the inner plate with an adhesive tape. The back side of the leaf was faced upwards. The cut end of the petiol was dipped in a bathing solution, into which the reference microelectrode was immersed. The measuring microelectrode was manipulated with a micromanipulator and inserted in an appropriate cell of the leaf.

Air or N_2 gas was introduced in the reaction vessel from the inlets (a) and (b) at the flow rate of 1.0～1.5 • min^{-1}. In order to obtain an anoxial condition, N_2 gas (purity 99.9999%) was introduced from the both inlets. When the leaf reacted with CO_2 gas, pure CO_2 gas(purity 99.99%) was introduced only from the inlet (a).

Initially the response to 100% CO_2 gas was recorded under the aerobic condition in the light. When CO_2 gas was supplied from the inlet (a), a remarkable change of leaf potential was obtained as shown in Fig. 6-①. After a transient vibrating mode, it reached about 30 mV more positive than the initial level. When CO_2 gas was replaced by air, the potential decreased sharply, then increased again and reached the initial level.

When 100% CO_2 gas was supplied from the inlet (a) in the dark, the amplitude of the response was no greater than 50% of Fig. 6-①. Then the leaf was illuminated again and air at the inlets (a) and (b) was replaced by N_2 gas. After the potential became another steady level, 100% CO_2 gas was supplied from the inlet (a). As shown in Fig. 6-③, the amplitude of the response was markedly reduced to about 20% of Fig. 6-①. Successively the light was shielded. After the potential became a steady level, 100% CO_2 gas was supplied again from the inlet (a). As shown in Fig. 4-④, no appreciable response was obtained.

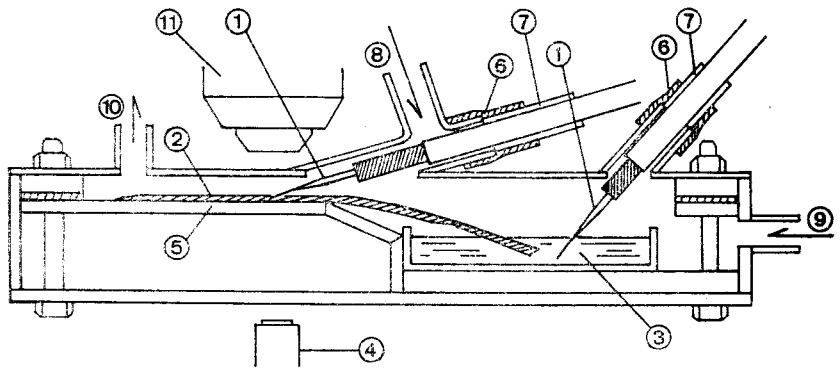

Fig. 5. Experimental setup for the measurement of leaf potential under an anoxial condition. ① microelectrode (Ag/AgCl), ② tobacco leaf, ③ bathing solution, ④ photoguide, ⑤ inner plate, ⑥ silicon tube, ⑦ electrode guide, ⑧ inlet (a), ⑨ inlet (b), ⑩ outlet, ⑪ microscope.

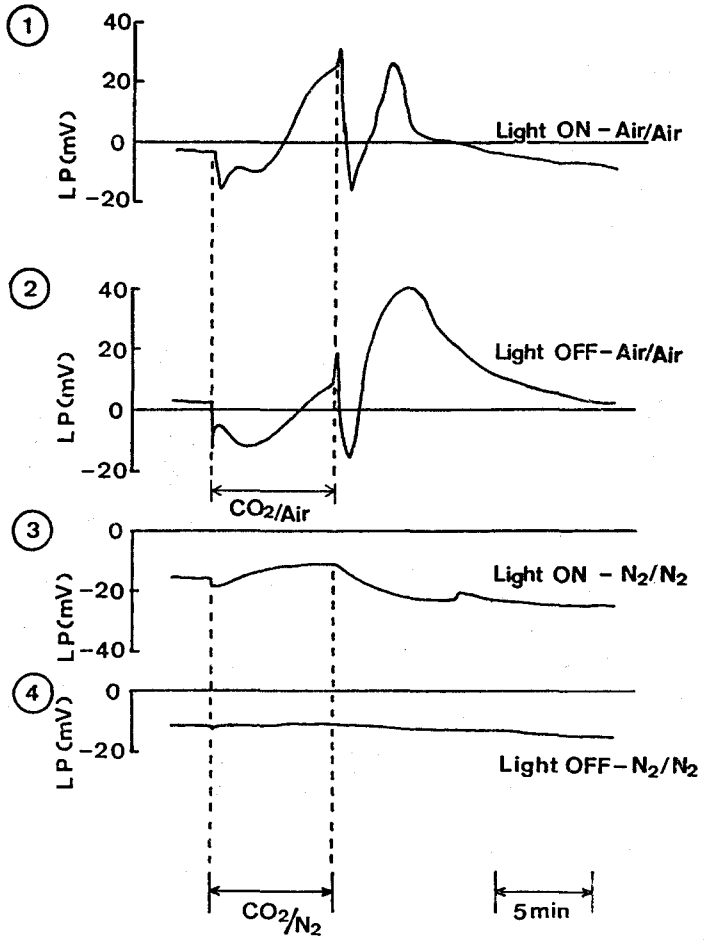

Fig. 6. Changes of leaf potential (LP) in response to the anoxial condition (N_2 gas) and/or the light shielding. Each profile was obtained from different leaves. X/Y means that gas X and gas Y are introduced from the inlets (a) and (b), respectively. ① Responses to the anoxial condition in the light. ② Responses to the light shielding under the aerobic condition. ③ Responses to the anoxial condition in the dark. ④ Responses to the light shielding under the anoxial condition.

Based on these results, the response to CO_2 gas can be ascribed to the electrogenic potential change. The energy is supplied mainly by aerobic respiration rather than by photosynthetic pathway.

4. Response Of A Plant Leaf To Odour Compounds

Various odour compounds were applied on a sample leaf of plant. There were some examples that a leaf responded to an odour compound directly by changing its leaf potential. A leaf of *L. stolonifera*, for instance, changed its leaf potential 5~10 mV in the negative direction in response to menthone or formaldeyde. On the other hand, 1, 2-dichloroethane caused a potential change in the positive direction of about 10 mV. Fig. 7 shows the residual effects of odour exposure on the response to CO_2 gas. When a sample leaf of *L. stolonifera* was exposed to CO_2 gas after the exposure to camphor or menthone, the response profile was remarkably altered, though the extent of alte-

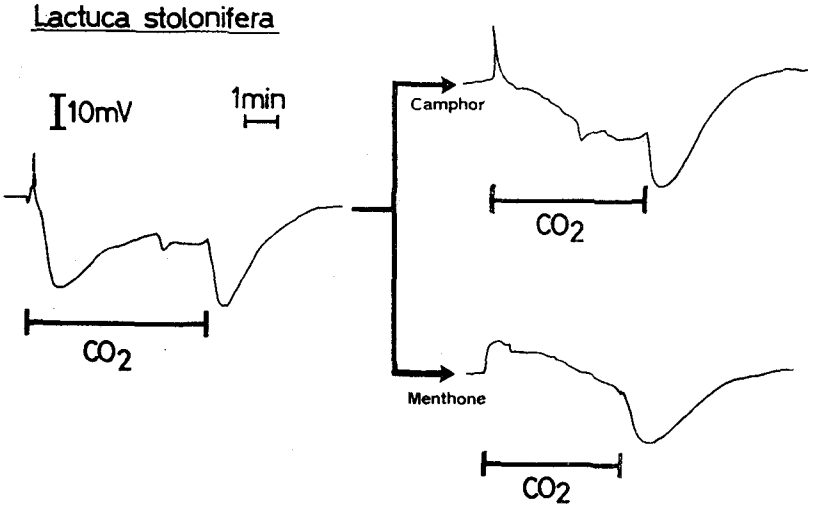

Fig. 7. Changes in response pattern of a *L. stolonifera* leaf to 100% CO_2 gas after the exposure of odour compounds. Initially the sample leaf was exposed to 100% CO_2 gas and response pattern was recorded. Then the leaf was exposed to air containing camphor or menthone. After that the leaf was exposed to CO_2 gas again and the response pattern was recorded.

ration was different in both cases[16].

That a plant leaf is actually sensing an odour compound can be more clearly recognized from the comparison of the response profile to CO_2 gas containing the odour compound with that to pure CO_2 gas. Fig. 8 shows a typical example obtained from a leaf of *G. jasminoides* in response to six compounds selected from jasmine-like odours (methyl dihydrojasmonate, α-amyl cinnamic aldehyde, benzyl acetate) and lavender-like odours(linalool, linalyl acetate, limone). Among three jasmine-like odours, benzyl acetate showed a most remarkable effect on the response to CO_2[17]. As depicted in Fig. 8, benzyl acetate apparently suppressed the response. In contrast, lavender-like odours

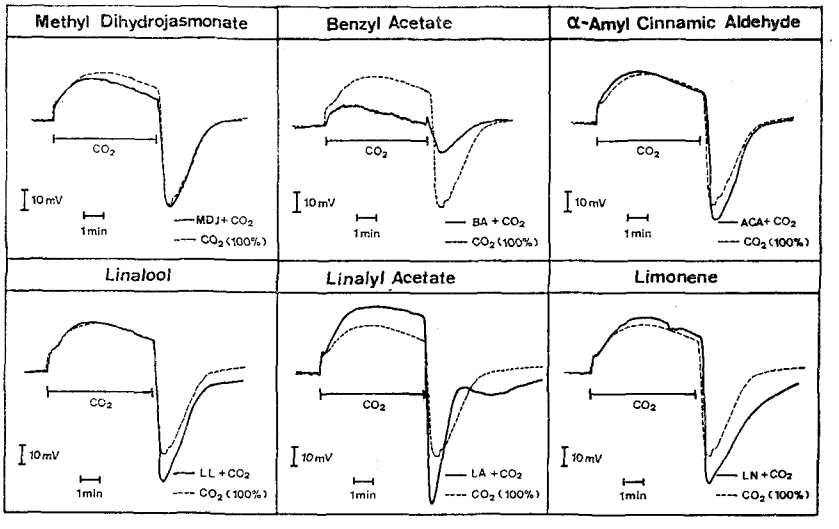

Fig. 8. Responses of *G. jasminoides* to CO_2 gas containing an ordour compound.

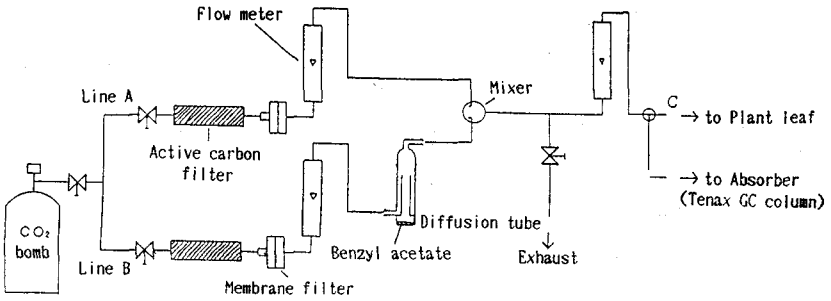

Fig. 9. Flow system for quantitative supply of odour compounds.

intensified the response to CO_2 gas. Among three compounds, lynalyl acetate was most effective. Jasmine-like odours are well known to have awaking and stimulating effects on human mentality, while lavender-like odours have calming and sedative effects. Our results were apparently opposite to those expected based on the analogy.

In order to study the quantitative response to an odour compound, it is necessary to develop a system for the quantitative supply of the odour compound. Fig. 9 shows a flow system using a diffusion tube. The diffusion tube was composed of odour reservoir, inner tube, and outer tube. An aliquot of odour compound was put in the reservoir. As a sample compound, benzyl acetate was used in this experiment. From the head of the diffusion tube, the odour compound came out and was diluted quantitatively with the carrier gas. The dilution rate was controlled by changing the flow rate of the carrier gas. The resulting gas was further diluted by mixing with the pure carrier gas to make a reaction gas. The reaction gas was sent to a sample leaf.

The actual concentration of odour compound in the reaction gas was checked separately as follows. The reaction gas was passed through a glass column containing adsorbent(Tenax GC or Tenax TA, AKZO Research Laboratories) for 15~20 min. Tenax GC column in which odour molecules were trapped and condensed was heated to release the odour molecules at once and to introduce them into gas chromatograph. The benzyl acetate concentration in the reaction gas was estimated 3.8~5.3 ppb, which was several hundred times lower than that directly detectable by gas chromatograph.

When CO_2 gas containing benzyl acetate was reacted, the response intensity was remarkably suppressed as described above. Especially, the respone obtained at the cut off of CO_2 gas was

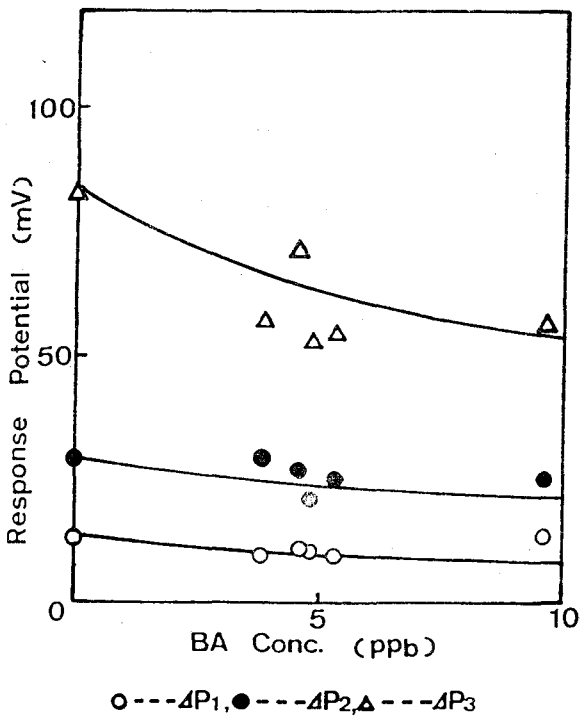

Fig. 10. Effect of benzyl acetate(BA) concentration on the response of a tobacco leaf to CO_2 gas containing BA.

suppressed. After flushing with air, the response to pure CO_2 gas was examined repeatedly. As shown in Fig. 10, the extent of the suppression increased as the benzyl acetate concentration increased in the range 0～9.8 ppb.

5. Use Of Plant In Biosensing

Responsive properties of a leaf cut from a plant body have been investigated and its promising properties have been well demonstrated. Their responses can be also obtained from a leaf attached on a plant body. Thus a whole body of plant can be used as an excellent material for chemical sensing. Moreover a whole body of plant should be superior to a cut leaf on the stability (life time). What to be done is only to combine an appropriate transducer with the plant to convert its response to an electric signal.

From the viewpoint of biosensing, we need not limit the plant function to chemical sensing. As illustrated in Fig. 11, plant may respond to physical or biological informations (i.e. stimuli) as well as to chemical informations. We can recognize the response of the plant from the change of its chemical, physical, biological or biochemical properties. Odour sensing described above is an example of the response to chemical informantion (odour compound). Its response is the change of the electrochemical properties detectable with an electrode.

The concept of biosensing might not be a new one. The physical check up and clinical analysis performed at hospitals may be categorized in biosensing of humans. However, a more important factor in the concept is that a whole living thing is used as an active component to recognize molecules and to emit corresponding signals. If a whole body of plant or animal is incorporated, it is possible to utilize such an excellent function as may hardly be expected from simple biomaterials isolated from the whole body or any artificial materials. The sensing function for odour compounds in the gas phase is considered such an excellent fune-

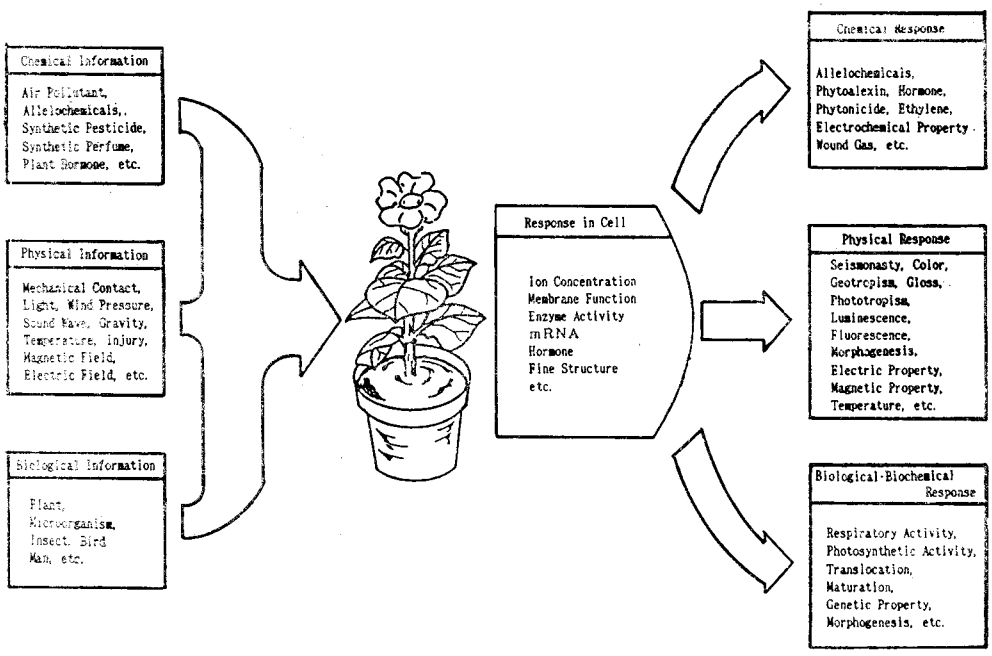

Fig. 11. Response of plant to various informations (stimuli).

tion. The sensor system that can detect directly odour compounds enables a none-invasive sensing for chemical compounds, which is considerably useful in chemical analysis.

6. Conclusion

Plant leaf or whole body of a plant is promising material for the biosensing for gaseous chemicals. Odour sensing at ppb level was actually possible with use of a leaf of *G. jasminoides* or *N. tabacum*. In the next step, it is necessary to improve the method for the quantitative analysis of odours. Our efforts have now been concentrated on this point.

About the biofunctions of plant, many things are still unresolved. In order to investigate their functions, it is essential to analyze the responses to respective informations. The development of transducing devices is also important. The more advanced and sensitive devices are available, the more precise plant informations can be obtained. Thus various novel and attractive sensor systems may be developed.

In conclusion, plant is promising material in sensor technology and it is important to investigate the potentiality of its novel functions.

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