

How to Apply Tricky Biological Mechanisms to Agricultural and Industrial Production

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By the end of the 21st century, the world population will surely far exceed the current 6.6 billion, threatening the essential requirements for life due to environmental deterioration and shortened food supply. To overcome this looming threat, we must develop new biotechnologies. There are so many known natural phenomena that we may have neglected, not perceiving them as blessings of nature. Many more remain unknown. We must examine each of them carefully since the many tricky and complicated mechanisms behind simple natural workings could provide us with attractive research targets. How then do we apply these complicated natural mechanisms to agricultural/ industrial production?

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Find Similarities in the Life Styles of Plants and Animals

For instance, a simple comparison of the metabolisms between plants and animals makes us clearly perceive many similarities. Since we as animals require organic compounds as energy sources, the following questions should be addressed.

- 1) Do plants take up organic compounds and metabolize them?
- 2) Do parasites infect plants and suppress the metabolic activity?
- 3) What types of circulation system do plants have?
- 4) How does the system work to convey water to the top of a tree over 30 m in height?
- 5) Are any physiological events in plants regulated by peptide hormones as in animals?

We have discovered that plants only need three major nutrients: nitrogenous and phosphorous compounds plus potassium ions. Of course, oxygen, carbon dioxide, water, some minerals, light, and a proper temperature are also essential for plant growth. While water is introduced from roots and some from leaves, carbon dioxide is generally taken up through the leaf stomata.

Is it Possible to Supply CO₂ through Roots?

Recently, we have found that plants readily take up formic acid, which is converted into CO₂ in tissues, and NADH is produced as a result of its degradation into CO₂. Strong light is not always favorable for photosynthesis. Light stress to

PSII *in vivo* becomes a problem for plants when the rate of photodamage exceeds the capacity of the repair process. There is now a consensus that the main targets for light stress in organisms possessing oxygenic photosynthesis are the PSI and PSII centers, leading to the impairment of electron transport, particularly in the so-called D1 protein. This stress situation, known as photoinhibition, is a physiological phenomenon caused by the reactive oxygen species produced as a result of excess excitation energy. In particular, photoinhibition is aggravated if this stress is combined with other stress factors, such as low temperature, drought or carbon dioxide deficiency, and inhibition can occur even at moderate light intensities. CO₂ deficiency in leaves is also caused by drought stress that induces stomata closing. Without the photosynthetic carbon reduction caused by CO₂ deficiency, utilization of the excitation energy is negligible while the interception of incoming light continues unabated, and this continued illumination may result in photoinhibition.

We have investigated the effect of potassium formate on the photosynthesis of rice under the photoinhibitory condition of CO₂ depletion. The application of formic acid before the photoinhibitory treatment lowered the photoinhibitory damages to PS II and photosynthetic carbon reduction enzymes, strongly suggesting that potassium formate has a protective effect against the photoinhibition of photosynthesis. This also suggests that endogenous application of formate can protect plants from photodamages occurring under strong light and drought conditions.¹⁻⁶⁾

It is well-known that composts originating from old leaves of trees and weeds can fertilize soil, and soil amendment by composts yields better crops. Several explanations have been provided for the soil amendment by composts leading to the fertilization of arable lands. One of the reasons is that composts improve soil structures, and the activities of microbes and soil worms are enhanced by the application of the composts to the cultivated soil.

But how do the degradation products from composts affect the growth of plants? We have found that phenolic compounds from the composts stimulate the root metabolism and induce the secretion of sugar and amino acids. Such root exudates are important for microbial activities in the soil and are very useful for the establishment of the proper rhizosphere of the individual plant species.

Degradation products from the composts, i.e., ferulic acid or *p*-coumaric acid, are readily taken up by plants and converted into hydroxystyrenes, which show strong antibacterial activities.⁷⁾ Other organic compounds such as common organic acids and amino acids are also taken up and integrated into the plants' own metabolism.

Strange Plants Which Can Eat Insects

Upon considering the uptake mechanism of organic compounds in the leaf, one may hit upon a unique plant type, the carnivorous plant, which absorbs the digestion products of specific organs regenerated from leaf tissues. How then do they digest the prey they capture?

The *Nepenthes* genus of carnivorous plants captures insects in trapping organ called pitcher and digest them in the pitcher fluid, which is strongly acidic and includes digestive enzymes. We found that pH of the fluid decreases when preys accumulate in the pitcher of *Nepenthus alata*. The pH decrease may be important for prey digestion and the absorption of prey-derived nutrients. We have identified several digestive enzymes in the pitcher fluid including phospholipase and proteases, which readily hydrolyzes a model peptide, insulin, into tri- and dipeptides and finally

into amino acids.⁸⁻¹⁰⁾ Furthermore, phosphatase activity of the fluid was high, suggesting that this enzyme plays an important role in assuring ample phosphorus supply from the insect components.

Understanding the problematic mechanisms of prey capture and digestion in carnivorous plants should provide us with several application insights. The uptake mechanism of organic compounds into plant tissues is not yet fully understood. Active transport mechanisms for digestion products are probably present even in the pitcher. The strength of uptake function of organic compounds in these leaf tissues could enable us to establish new culture methods for garden vegetables without roots.

New Sources of Energy and Raw Materials Replacing Fossil Fuels

Recently, environmental deterioration has become a serious problem. During the past few decades, we have consumed large amounts of fossil fuels which increased the carbon dioxide content of the air by as much as 30% from the level of about 100 years ago, and, as a result, the earth

has encountered many unpredictable problems. Overproduction of carbon dioxide has meant that in less than past 100 years we have used up more than half of the fossil fuel deposits, built up over billions of years. The biosphere in which we live has been established over a long period of time. To remedy the polluted environment, we must suppress the consumption of fossil fuels and find alternate energy sources.

These fossil fuels have been used as not only energy sources but also as sources of industrial raw materials. However, before we began to use fossil fuels, plants had been the major sources of both. Thus, terpenes and fatty acids derived from plants should be promising substitutions for the industrial raw materials.

Among terpenes, rubber is a unique biomaterial. The component molecules in rubber are polyprenols in which isoprenoid units are polymerized to give linear polymers of varying degrees of polymerization. Isoprenoid units are synthesized through two biosynthetic routes, the mevalonic and Rohmer's pathways. It is critical to determine the major pathway for rubber biosynthesis. If we can understand the complete mechanisms of prenyl polymerization, we may reconstruct the polymerization process as an *in vitro* system on an industrial level. Allergy due to proteinous contaminants in rubber products is a serious problem. It should be possible to remove the antigens *in vitro* from the rubber products.

Metabolic Engineering in Plants

From the viewpoint of metabolic engineering, enforcement of the biosynthetic pathways of isoprenoids such as isoprene, farnesol, and geraniol, among others, ensure a large accumulation of polyprenols in plant.

We are most interested in plants that can grow in the Temperate Zone and also possess the potential to produce rubber.^{11,12)} Many Compositae and Euphorbiaceae plants are known to produce polyprenols. However, these have not been used as rubber sources since their productivities of polyprenols are very low.

If we are forced to limit the domestic use of fossil fuels in the near future, plants will become not only key sources for energy, but plant-origin polyprenols and fatty acids will become major sources of raw materials to replace chemical products made from fossil fuels.

Plant utilization research must be entrusted to the hands of researchers not only in the agricultural fields but also in the engineering fields. It may thus be said that now is the time the new unique research concepts of different fields should be combined and oriented towards GREEN CHEMISTRY and GREEN RIVER projects to protect our lives from the polluted environment.

References

1. Shiraishi, T., Fukusaki, E., Kajiyama, S. and Kobayashi, A. (1999) A simple assay for formate dehydrogenase activity by gas chromatography-mass spectrometry. *J. Chromatogr. A* **855**, 337-340.

2. Shiraishi, T., Fukusaki, E. and Kobayashi, A. (2000). Formate dehydrogenase in rice plant: Growth stimulation effect of formate in rice plant. *J. Biosci. Bioeng.* **89**, 241-246.
3. Shiraishi, T., Fukusaki, E., Miyake, C., Yokota, A. and Kobayashi, A. (2000) Formate protects photosynthetic machinery from photoinhibition. *J. Biosci. Bioeng.* **89**, 564-568
4. Fukusaki, E., Shiraishi T., Kajiyama, S. and Kobayashi, A. (1998) Effect of formate on the growth of rice (*Oryza sativa*). Abstract of the annual meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry **72**, 169.
5. Shiraishi, T., Fukusaki, E., Kajiyama, S., Okazawa, A. and Kobayashi, A. (1998) Effect of Cl compounds on the plant metabolism: Growth stimulation by formate in rice plant. Abstract of the 33th annual meeting of the Japanese Society for *Chemical Regulation of Plants*, 29-30.
6. Shiraishi, T., Fukusaki, E., Nishikawa, T., Miyake, T., Yokota, A., Kajiyama, S., Okazawa, A. and Kobayashi, A. (1999) Effect of formate on the photooxidative damage in rice. Abstract of the annual meeting of Japan Society for *Bioscience, Biotechnology, and Agrochemistry* **73**, 362.
7. Kobayashi, A., Kim, M. J. and Kawazu, K. (1996) Uptake and exudation of phenolic compounds by wheat and antimicrobial components of the root exudate. *Z. Naturforsch.* **51c**, 527-533.
8. An, C. I., Fukusaki, E. and Kobayashi, A. (2000) Plasma-membrane H⁺-ATPases are expressed in pitchers of the carnivorous plant *Nepenthes alata* Blanco *Planta* (in press).
9. Fukusaki, E., An, C. I., Kajiyama, S. and Kobayashi, A. (1998) Digestive enzymes from the carnivorous plant *Nepenthes alata*. Abstract of the annual meeting of Japan Society for *Bioscience, Biotechnology, and Agrochemistry* **72**, 169.
10. Fukusaki, E., An, C. I., Kajiyama, S., Okazawa, A. and Kobayashi, A. (1999) cDNA cloning of plasma membrane proton pump of the carnivorous plant *Nepenthes hybrida*. Abstract of the 34th annual meeting of the Japanese Society for *Chemical Regulation of Plants*, 165-166.
11. Bamba, T., Fukusaki, E., Kajiyama, S., Okazawa, A., Ute, K., Kitayama, T. and Kobayashi, A. (2000) Elucidation of polyisoprene biosynthesis in plants. Abstract of the annual meeting of Japan Society for *Bioscience, Biotechnology, and Agrochemistry* **74**, 2.
12. Bamba, T., Fukusaki, E. and Kobayashi, A. (2000) High-resolution analysis of polyprenols by super critical fluid chromatography. *J. Chromatogr.* (submitted).