Introduction
Plants are continuously exposed to attacks from various enemies; however, as plants cannot move, they have to defend themselves from the ground. They have therefore evolved a secondary metabolism to synthesize various types of defense substances in response to external stresses caused by pathogenic microbes or physical wounding. Such defense substances have been attracting the attention of many researchers, not only as possible lead compounds to develop new antifungal or other biological active agents, but also as tools to investigate the molecular mechanisms of inducible defense responses as well as the recognition of nonself to trigger them in plants.

The involvement of terpenoids and phenolic compounds derived from phenylalanine in plant chemical defense is well established. In addition, it is often observed that characteristic nitrogen-containing secondary metabolites of various amino acid origins are induced to be accumulated in plant tissues under stressed conditions; however, their chemistry, induction mechanism and biosynthetic regulation, and even their role in defense are relatively unelucidated, and thus are quite intriguing. Here we briefly introduce the results of our research into the nitrogen-containing substances associated with defense and stress responses in plants.

1. Aromatic amines and amides induced in response to external stresses
In oats, a gene-for-gene relationship has been observed concerning cultivar-specific resistance toward crown rust fungus, and the accumulation of phytoalexin in leaf tissues interacting with pathogens has been demonstrated to play an important role in rejection of the pathogen by the plant. The phytoalexin of oats was identified to be a series of amide compounds referred to as avenanthramides, which are composed of p-coumaric acid derivative and hydroxyanthranilic acid. While the former is frequently found as a component of many plant secondary metabolites with defensive functions, the latter is derived from anthranilate, an important biosynthetic intermediate of tryptophan, found rather specifically. The biosynthesis of avenanthramides in oats is not only induced by infection with the pathogen, but also by an oligomer of chitin, a major polymer component of fungal cell walls. Oat plants are likely to recognize such a fragment of chitin as the molecular pattern of non-self.

In barley, UV irradiation of leaves induced a marked accu-
mulation of tryptamine, probably derived from tryptophan by decarboxylation. Although its role in defense against pathogens was unclear in barley, a marked accumulation of hydroxylated tryptamine, or serotonin was also observed in the leaf tissues of rice interacting with brown spot fungus, Bipolaris oryzae. The accumulation of serotonin was particularly evident in lesioned tissues, and was closely associated with the brown appearance of leaf spots. Serotonin was considered to be converted to an insoluble brown material in the cell walls of leaf tissues at the infection sites to function as a physical barrier to invasion by the pathogen. Taking these and other examples in the literature into consideration, upregulation of the tryptophan pathway appears to be importantly involved in the defense responses of gramineous plants.

In potato, the treatment of tuber tissues with an elicitor prepared from the pathogenic fungus of potato late blight, Phytophthora infestans, caused marked accumulation of a phenolic amide compound, p-coumarylooctopamine (pCO). The component, octopamine, is a biogenic amine synthesized from tyrosine via decarboxylation and subsequent oxidation. Antifungal activity of pCO is almost negligible, but is incorporated into cell walls to form polymeric barrier materials for defense. In contrast with oat, the accumulation of pCO in potato tuber tissues is induced by β-1,3-glucooligosaccharides, including laminarin from brown alga Laminaria digitata. Treatment with glucooligosaccharide elicitors elevates the activity of enzymes responsible for biosynthesis, such as phenylalanine ammonia lyase, 4-hydroxyxynamic acid:CoA ligase, hydroxycinnamoyl-CoA:tyramine N-(hydroxycinnamoyl)transferase and tyrosine decarboxylase. Among these, the response of tyrosine decarboxylase was specific to elicitor treatment, and its regulation appeared to be critical for the accumulation of pCO.

2. Metabolic flux analysis for the biosynthesis of phenolic compounds in potato tuber tissue

In general, the expression levels of genes encoding enzymes involved in the biosynthesis of a metabolite or even the levels of enzyme activity themselves do not account for the actual activity of a biosynthetic pathway for the metabolite or the amount of metabolite found in the tissues. To evaluate the exact biosynthetic activity, it is necessary to monitor material flow in the pathway, which is referred to as metabolic flux defined as the amount of metabolite converted per specified time unit. Accordingly, we tried to obtain metabolic flux values for biosyntheses of pCO and structurally-related components in potato tuber tissues in order to understand the situation of metabolite production.

Deuterium-labeled phenylalanine (Phe) was administered in disks prepared from potato tuber tissue, and time-dependent incorporation into pCO was monitored using LC-MS. For the conversion from Phe to pCO, the isotopic abundance of pCO ($C_{pCO}$) is related to the time after the supply of Phe with isotopic abundance of $C_{Phe}$ as follows:

$$C_{pCO} = C_{Phe} \left[ 1 - \frac{V(0)}{v + V(0)} \right]$$

In this equation, $J_{in}$ is the flux from Phe to pCO and $V(0)$ is the pool size of pCO at $t=0$. Parameter $v$ is defined as the difference between $J_{in}$ and $J_{out}$ (flux from pCO to its conversion product), and deemed as constant under the conditions in which the amount of pCO is linearly increased. The value of $J_{in}$ is obtained by fitting the time-dependent change in $C_{pCO}$ to this equation, using the experimentally determined $v$.

The fluxes of formation ($J_{in}$) and conversion ($J_{out}$) of pCO were determined as 1.15 and 0.96 nmol/g FW/h, respectively, in injured potato tuber tissue, where no apparent accumulation of pCO was observed, and therefore, demonstrating that the absence of a compound does not necessarily mean the absence of its production. On the other hand, those for chlorogenic acid, one of the most major components in potato tuber, were 4.63 and 0.42 nmol/g FW/h, respectively, accounting for its occurrence at a high level in the tissue.

Treatment of the tuber disks with glucooligosaccharide elicitor caused an increase in the flux of formation for pCO by about 10-fold, while that for chlorogenic acid was somewhat decreased. In contrast, flux values of conversions of pCO and of chlorogenic acid were both increased about 3-fold. A significant difference between fluxes of the formation and conversion of pCO resulted in its marked accumulation, while a larger flux of conversion caused a decrease in the level of chlorogenic acid. Flux analysis demonstrated that some metabolites are produced and converted more actively than it appears from snapshots of metabolite composition at certain points in a tissue, and revealed the importance of pCO as a component of defense reactions in potato tuber tissue.

3. Regulation of primary and secondary metabolism associated with tryptophan biosynthesis

Many gramineous plants have defense substances that are associated with tryptophan biosynthesis, such as serotonin in rice, avenanthramides in oats and benzodioxazinones in maize and wheat. Thus, it is anticipated that the activation of tryptophan biosynthesis may result in improved tolerance to diseases and pests, as well as nutritional value as a source of the essential amino acid. To date, many breeding trials to raise tryptophan contents in crop plants have been performed, and it has been demonstrated that utilization of the OASAI D gene, which encodes the mutant anthranilate synthase α-subunit with reduced feedback sensitivity to tryptophan, is extraordinarily effective, giving rise to an increased amount of Trp by 2- to 300-fold in several plants, including rice. This provides a good opportunity to investigate the effects of Trp overproduction on the composition or profile of secondary metabolites related to anthranilate/tryptophan. Comprehensive metabolic profiling analysis using HPLC-UV and -MS techniques re-
revealed, however, that activation of the Trp biosynthetic pathway by the overexpression of *OASA1D* caused no obvious change compared to untransformed plants, except for the marked increase in the amount of Trp. Thus, it was demonstrated that overproduced Trp was isolated in some cell compartments and was strictly regulated to prevent flow into the secondary metabolism.

In contrast, metabolic profiling analysis of the rice *mtr1* mutant that accumulates a higher amount of Trp revealed an interesting aspect of the regulation of secondary metabolism. In addition to Trp, the mutant accumulated higher levels of phenylalanine and tyrosine, the biosynthesis processes of which share a common shikimate pathway with Trp, and the accumulation of phenylalanine-derived secondary metabolites was also observed. Intensive genetic analysis clarified that the *mtr1* mutant had an amino acid substitution in arogenate dehydratase, one of the enzymes that constitute the phenylalanine biosynthetic pathway, and this substitution gave rise to a reduction of feedback inhibition by phenylalanine, and consequently, elevation of the phenylalanine level. The mechanism of increase in the level of Trp by enhanced biosynthesis of Phe is unknown and will be an absorbing subject of future research. This result also highlighted the intriguing difference in the regulation of Phe-derived secondary metabolism, compared to that derived from Trp: the former relatively easily enables the utilization of overproduced Phe in the production of the related secondary metabolites, while the latter is under strict regulation and converts only a limited fraction of excess Trp into downstream metabolites.

**Conclusion**

Nitrogen-containing secondary metabolites originate from amino acids in most cases via the decarboxylation reaction. This type of reaction can form a counterpart with the deamination reaction of phenylalanine to yield a number of phenolic secondary metabolites, the role of which in plant defense has been well established. Regulation of the decarboxylation of amino acids and its relevance to defense mechanisms in plants are relatively unelucidated. Further studies will provide many interesting targets in the future for new chemical methods of plant growth control as well as the enhancement of disease resistance.