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学位論文要旨

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題目: Constitutive and inducible defensive metabolites in Hordeum species and wheat
（オオムギ属植物とコムギにおける構成的および誘導性防御物質に関する研究）

Plants possess chemical defense systems to protect themselves against biotic stresses. Antifungal compounds in plants, such as phytoanticipins and phytoalexins, contribute to the rejection of pathogen infection. Phytoanticipins are defined as low molecular weight, antimicrobial compounds that are present in plants before challenge by microorganisms or are produced after infection solely from preexisting constituents. On the other hand, phytoalexins are defined as low molecular weight, antimicrobial compounds produced by plants in response to pathogen attack, and provide a chemical barrier against the invasion of pathogen.

In general, closely related species accumulate similar specialized metabolites. However, in the genus *Hordeum* (Poaceae), which includes cultivated barley (*Hordeum vulgare* ssp. *vulgare*), the occurrence of multiple defensive metabolites such as hordatines, benzoxazinones (Bxs), and gramine has been reported. *Hordeum* species are classified into four clades, H, Xu, Xa, and I. The H and Xu clades comprise a monophyletic group, while the I and Xa clades form a separate monophyletic group. However, the correlation between phylogeny and the distribution of defensive specialized metabolites in *Hordeum* has not been clarified. To reveal the evolutionary changes in secondary metabolism in the genus *Hordeum*, the presence or absence of defensive specialized metabolites was analyzed in representative *Hordeum* species in all four clades. In the H clade, *Hordeum vulgare* accumulated hordatines but not Bxs, whereas *H. bulbosum* accumulated neither compound. *H. vulgare* ssp. *vulgare* ‘Shurai’, *H. vulgare* ssp. *spontaneum*, and *H. murinum* ssp. *leporinum* accumulated gramine at high concentrations, while several species in I clade also accumulated it at low concentrations. Species in the clades I and Xa accumulated Bxs without hordatines. In *H. murinum*, a Xu clade species, neither hordatines nor Bxs were detected. Two hitherto undescribed compounds were found to commonly accumulate in *H. bulbosum* in the H clade and in *H. murinum* in the Xu clade. On the basis of spectroscopic analyses, they were identified as dehydrodimers of feruloylagmatine and were designated murinamides A and B. These compounds showed antifungal activities against pathogenic fungi, indicating their defensive roles. As hordatines are also dehydrodimers of phenylamides with agmatine, both the H and Xu clade species are considered to accumulate the same class of compounds. Thus, when the H/Xu clades split from the I/Xa clades during evolution, the defensive metabolism shifted from the synthesis of Bxs to the synthesis of dehydrodimers of phenylamides with agmatine plus gramine in the H/Xu clades.

Plants often activate multiple specialized metabolic pathways upon pathogen infection. Poaceae species such as rice and maize accumulate phytoalexins with diverse chemical structures including phenylamides, flavonoids, and terpenoid. On the other hands, in barley and wheat, inducible defensive systems with specialized metabolites remain to be elucidated while representative phytoanticipins such as hordatines and Bxs, respectively, have been identified. Changes in specialized metabolites were analyzed in wheat leaves inoculated with *Bipolaris* *sorokiniana*, the causal agent of spot blotch in *Poaceae* species. HPLC analysis detected the accumulation of six compounds in *B. sorokiniana*-infected leaves. Of these, we purified two compounds by silica gel and ODS column chromatography and preparative HPLC, and then identified them as cinnamic acid amides, *N*-cinnamoyl-9-hydroxy-8-oxotryptamine and *N*-cinnamoyl-8-oxotryptamine, by spectroscopic analyses. We named these compounds triticamides A and B, respectively. The remaining four compounds were predicted to be *p*-coumaric acid amides of hydroxyputrescine, hydroxyagmatine, hydroxydehydroagmatine, and agmatine by mass spectrometry. To examine the localization of triticamides A and B in *pathogen*-inoculated leaves, lesioned and healthy tissues were extracted separately and subjected to HPLC analysis. Triticamides were detected at high concentrations in the lesioned tissue of the leaves. The accumulation of two cinnamic acid amides was also induced by *Fusarium graminearum* infection, and by treatment with $CuCl_2$, jasmonic acid, and isopentenyladenine. Antifungal activity of these amides was demonstrated by inhibition of conidial germination and germ tube elongation of pathogenic fungi, such as *Fusarium graminearum* and *Alternaria brassicicola*, thus indicating that they act as phytoalexins. The accumulation of these amides was also detected in barley leaves treated with $CuCl_2$. We examined the accumulation of 25 phenylamides in *B. sorokiniana*-infected wheat leaves using LC-MS/MS. Hydroxycinnamic acid amides of tryptamine, serotonin, putrescine, and agmatine were induced after infection with *B. sorokiniana*. Thus, the induced accumulation of two groups of phenylamides, cinnamic acid amides with indole amines and *p*-coumaric acid amides with putrescine- and agmatine-related amines, represents a major metabolic response of wheat to pathogen infection.

Metabolic changes in pathogen-infected barley were examined in addition to those in wheat. HPLC analysis detected the induced accumulation of three compounds in barley roots challenged by *Fusarium culmorum*, the causal agent of Fusarium root rot. The three compounds were identified as triticamides A and B, and *N*-cinnamoyl-(1*H*-indol-3-yl)methylamine, which we named triticamide C, by spectroscopic analysis. Triticamides A and B were also detected in wheat, whereas triticamide C was detected only in barley. On the basis of their antimicrobial activities, triticamides function as phytoalexins in barley. The accumulation of 25 phenylamides in pathogen-infected root and leaves were also examined using LC-MS/MS. Accumulation of phenylamides with putrescine, agmatine, tyramine, tryptamine, and serotonin was induced in the pathogen-infected barley. The administration of deuterium-labeled *N*-cinnamoyl tryptamine (CinTry) to barley roots resulted in the effective incorporation of the compound into triticamides A and B, which suggested that they were synthesized through the oxidation of CinTry. Nine putative tryptamine hydroxycinnamoyl transferase (THT)-encoding genes (*HvTHT1–HvTHT9*) were identified by database search on the basis of homology to known THT gene sequences in rice. Since *HvTHT7* and *HvTHT8* had the same sequences except one nucleotide, we measured their expression levels in total by RT-qPCR. *HvTHT7/8* were markedly upregulated in *F. culmorum*-infected root and *B. sorokiniana*-infected leaves. The HvTHT7 and HvTHT8 enzymes preferred cinnamoyl- and feruloyl-CoAs as acyl donors and tryptamine as an acyl acceptor, and (1*H*-indol-3-yl)methylamine was also accepted as an acyl acceptor. These findings suggested that HvTHT7/8 are responsible for the induced accumulation of triticamides in barley.

In the present study, we found that barley and its related species in H and Xu clades in the genus *Hordeum* accumulate HCAA dimers as phytoanticipins, whereas the species in I and Xa clades accumulate Bxs in common with wheat. This distribution of different classes of compounds suggests great metabolic changes that occurred in the evolution of species in H and Xu clades. On the other hand, barley and wheat accumulated common phenylamide phytoalexins, triticamides, in response to pathogen attacks. As these compounds have not been described in other species to the best of our knowledge, triticamides are considered to be characteristic compounds in these species. These differences and similarities of defensive specialized metabolites among Triticeae species are considered to be a reflection of “scrap-and-build” in specialized metabolism, which has occurred in evolution of Triticeae species for survival under varying biological stresses.