論文要約（４（２）関係）

博士論文要約（Summary）

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タイトル	Clarification of the mechanism underlying host-plant manipulation by gall-inducing insects （虫えい形成昆虫による寄主植物操作メカニズムの解明）

キーワード（Barley）（Cicadellidae）（insect-plant interaction）（wheat）（Poaceae）（rice）

Chapter 1. General introduction

Interactions of herbivores with their host plants play important roles in the evolution of their various traits. Approximately 13,000 herbivorous insects have an ability to manipulate their host plant tissue and induce galls for their own benefit. Although many researchers have been paid gall habits and its adaptive significances, the detailed mechanism underlying gall induction by insects is still unclarified. This is fundamentally owing to the difficulty in establishing mass rearing systems for most gall-inducing insects. The aim of this thesis is to clarify the host manipulation by gall inducers using the maize orange leafhopper *Cicadulina bipunctata* associated with poaceous plants. This species induces gall symptom characterized by stunted growth and swollen leaf veins on various Poaceae including important crops such as maize *Zea mays* L., rice *Oryza sativa* L.and wheat *Triticum aestivum* L. This leafhopper is an ideal study material to study the gall-inducing mechanism because the mass-rearing technique has already been established and model plants of Poaceae are readily available as hosts. Moreover, because *C. bipunctata* is an important pest of poaceous crops, clarification of gall-inducing mechanism is useful to develop novel resistant cultivars. In this thesis, I studied following aspects: (1) Distribution of *C. bipunctata* in Japan and its geographical variation in gall-inducing ability; (2) Oviposition-host preference and developmental performance of *C. bipunctata* on galled and non-galled host plants; (3) Effects of barley (*Hordeum vulgare* L.) chromosome addition on the susceptibility of wheat to feeding by *C. bipunctata*; (4) Varietal differences in susceptibility to gall induction by *C. bipunctata* in rice; and (5) Detection of rice genes related to gall induction by *C. bipunctata*. Then based on the results, I discussed the underlying mechanism of gall induction by *C. bipunctata*.

Chapter 2. Distribution of *C. bipunctata* in Japan and its geographical variation in gall-inducing ability

Although *C. bipunctata* is reported from the southern parts and middle-western parts of Kyushu, the detailed distribution in Honshu, Shikoku, and the northern parts of Kyushu was not examined

yet. Because the annual mean temperature in Shikoku and the Kii Peninsula, Honshu, is rather higher than that in the middle parts of Kyushu, *C. bipunctata* has possibilities to inhabit these areas. To assess the potential risk of agricultural damage by *C. bipunctata*, information about the detailed distribution of *C. bipunctata* and its gall-inducing ability in local populations will be very important. In this chapter, I surveyed the distribution of *C. bipunctata* in the southwestern and eastern parts of Shikoku and the southwestern part of the Kii Peninsula, and I compared the gall inducing ability of *C. bipunctata* between Kyushu and Shikoku populations. Field investigations revealed that *C. bipunctata* is distributed in Ehime and Kochi Prefectures of Shikoku as well as in middle-eastern parts of Kyushu. This is the first collection record of this species from Shikoku. In addition, the Kochi population is similar to the Kumamoto population in the ability to induce growth stunting and leaf vein swellings on susceptible and resistant varieties of maize. The present results clearly indicate that *C. bipunctata* has a potential to become a serious pest of poaceous crops in Shikoku as in Kyushu. Further attention will be needed to monitor the distribution range expansion of *C. bipunctata* populations in Japan.

Chapter 3. Oviposition-host preference and developmental performance of *C. bipunctata* on galled and non-galled host plants

In herbivorous insects, oviposition preference by females affects the development and survival of their offspring. Both positive and negative linkages between oviposition preference and offspring performance have been reported for many species. Although gall induction by *C. bipunctata* is known to improve nymphal performance, the oviposition preference of females between galled and non-galled host plants was not studied. In this chapter, the nymphal performance and oviposition and feeding sites preference of *C. bipunctata* were investigated using the galled and non-galled host plants, wheat and barley, respectively. As a result, the survival rate of *C. bipunctata* was significantly higher on wheat than on barley. Although in the choice test significantly more eggs were laid into barley, which had larger leaves than wheat, no significant difference was detected in the no-choice test. In addition, the number of remaining individuals per leaf area was not significantly different between wheat and barley, suggesting no clear oviposition preference by *C. bipunctata* between these plants. The nymphal experience of growing host did not affect female oviposition preference. The inconsistent correspondence between offspring performance and oviposition preference in *C. bipunctata* may reflect its high mobility of nymphs. The results show that as in some free-living herbivoures, oviposition preference and offspring performance are not always positively correlated in gall-inducing insects.

Chapter 4. Effects of barley chromosome addition on the susceptibility of wheat to feeding by *C. bipunctata*

Unlike other poaceous plants, barley does not exhibit any symptoms even when it was fed on by *C. bipunctata*. This implies that barley possesses certain genetic factors inhibiting the gall induction

in response to the feeding by *C. bipunctata*. Utilizing the high cross compatibility of *T. aestivum*, several wheat–barley chromosome addition lines have been established up to the present. They are useful for clarifying the genetic factors of barley inhibiting the growth stunting and gall induction by *C. bipunctata* and developing novel resistant cultivars to the leafhopper. To clarify the mechanism of growth stunting and gall induction by *C. bipunctata*, I used six barley chromosome disomic addition lines of wheat (2H–7H) and investigated the effect of barley (cultivar Betzes) chromosome addition on the susceptibility of wheat (cv. Chinese Spring) to feeding by the leafhopper. As a result, feeding by *C. bipunctata* significantly stunted the growth in 2H, 3H, 4H, and 5H, but did not in 6H and 7H. The degree of gall induction was significantly weaker and severer in 3H and 5H than in Chinese Spring, respectively. These results suggest that barley genes resistant to growth stunting and gall induction exist in 6H and 7H, and 3H, respectively. Then 5H is considered to be useful for future assays investigating the mechanism of gall induction by this leafhopper because of the high susceptibility to the feeding by *C. bipunctata*. Significant correlation between the degrees of growth stunting and gall induction was not detected in the six chromosome addition lines and Chinese spring. This implies that these two symptoms are independent phenomena although both are initiated by the feeding of *C. bipunctata*.

Chapter 5. Varietal differences of rice in susceptibility to gall induction by *C. bipunctata*

Genetic basis of gall induction has been seldom reported in plant-insect systems, because of the difficulty in comparing the degree of gall induction on model plant species. Among host plants of *C. bipunctata*, rice especially contains a huge number of cultivars for comparative experiments of genetic divergence. In this chapter, I comprehensively analyzed the susceptibility to gall induction by *C. bipunctata* among rice cultivars using a set of diverse rice germplasms from the world rice core collection (WRC). This core collection is suitable for investigating the genetic basis of complex traits, because they include representative varieties of almost 32,000 cultivated rice varieties. Among 61 cultivars of WRCs, seven cultivars, Calotoc (WRC22), Basilanon (WRC44), Ma sho (WRC45), Khao Nok (WRC46), Khau Mac Kho (WRC48), Padi Perak (WRC49), Rexmont (WRC50), and Phulba (WRC67), revealed to be resistant to *C. bipunctata*. This result implies that these rice cultivars have resistant genes to gall induction by the leafhopper. Although no cultivated lines of resistant rice to *C. bipunctata* is available for experiments at present, further screening using galling and non-galling cultivars discovered in this study will be useful to clarify the detailed information of loci related to gall induction and growth stunting by *C. bipunctata*. The result will also contribute to development of novel cultivars of poaceous crops resistant to infestation by *C. bipunctata*. In addition to cultivars in WRC, the susceptibility to gall induction by *C. bipunctata* were also studied in seven cultivars, Taichung 65, Nipponbare, Asominori, Kinmaze, IR24, Kasalath, and ARC10313. These seven cultivars are useful to reveal the genetic basis of several traits because various lines substituted by a part of different genes derived from other cultivars are available for analysis. Feeding experiments revealed that the gall induction on ARC10313 and Taichung 65 was highest and lowest among seven rice cultivars. Thus the two cultivars are useful

to reveal the genetic basis of several traits, because various lines substituted by a part of different genes derived from other cultivars are available for analysis (see Chapter 6 below).

Chapter 6. Detection of genes related to gall induction by *C. bipunctata*

At present, chromosome segment substitution lines (CSSLs) derived from a part of ARC10313 in the genetic background of Taichung 65 were available for various experiments. CSSLs are convenient for the analysis of the genetic basis of specific traits in the donor cultivars, because the other parts are uniform genetic background of substituted cultivars. Based on the comparisons of susceptibility to gall induction by *C. bipunctata* among TA-CSSLs, the high intensity of gall induction was affected by several loci in rice chromosomes 3, 6, 8, and 9. Totally 20 loci related to phytohormones were detected in these chromosomes, among which 14 loci were related to induction of auxin proteins. Auxins and cytokinins may play an important role for the gall induction, because almost all galls grow via hypertrophy and hyperplasy. In the case of *C. bipunctata*, abscisic acid (ABA) and trans-Zeatin (tZ) are clarified to significantly increase and GA_1 and GA_4 decrease in galled tissues, respectively. Further studies will contribute to understand the regulation mechanism and genetic molecular basis of gall induction by *C. bipunctata*. This study is valuable for not only the clarification of detailed mechanism underlying gall induction by insects but also the development of novel cultivars of poaceous crops resistant to infestation by *C. bipunctata*.