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REVIEW

Genetic Study on Resistance to the Common Cutworm and Other Leaf-eating Insects in Soybean

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Abstract

The common cutworm (*Spodoptera litura* Fabricius) is a major pest of soybean [*Glycine max* (L.) Merr.] in southwestern Japan, and other lepidopteran insects damage soybean crops in the United States. Plant resistance to these insects can contribute to integrated pest management. To develop soybean cultivars with insect resistance, resistant germplasms have been identified and used as resistance donor parents. The resistance conferred by their genes has been studied from genetical, morphological, and physiological perspectives. The morphological and physiological approaches have succeeded to some degree, but the main cause of the resistance remains unknown. However, genetic studies have made progress since molecular biological approaches became possible in soybean. Two quantitative trait loci (QTL) for the common cutworm resistance and 23 QTL for other leaf-eating insect resistance have been detected. Actual effects of the major QTL have been confirmed using near-isogenic lines. This progress in genetic studies of the resistance enables the development of elite soybean cultivars with insect resistance, despite the poor agronomic characteristics of resistance donor parents. The present review summarizes the recent progress in resistance to the common cutworm and other insects in soybean.

Discipline: Plant breeding / Insect pest
Additional key words: backcrossing, crop pests, DNA markers, genome information, QTL

Introduction

In southwestern Japan, the common cutworm (CCW; *Spodoptera litura* Fabricius) is one of the major insect pests of soybean[9,37]. We estimate that more than 80% of soybean fields were attacked by the CCW in southwestern Japan in 2008. It is common to apply insecticide two or three times per year to control CCW infestations in this area. We estimate that this costs farmers at least ¥8 billion per year. In addition it is obvious that the insecticide application is also a load to the natural environment.

To reduce the need for insecticide application and improve management of the insect, CCW-resistant soybean cultivars should play an important role. However, transgenic cultivars are not acceptable to Japanese consumers, so it will not be possible to develop resistant cultivars by means of genetic engineering (e.g., to introduce the *Bt* gene to crops). Thus, increasing the resistance of soybean plants to the CCW has been a key factor in current breeding programs for southwestern Japan. To assist such programs, researchers have searched for and identified CCW-resistant germplasms[12]. These plants were used as resistance donor parents, and the mechanisms of resistance have been investigated from morphological and physiological perspectives[11,12]. However, it has proven difficult to develop resistant cultivars that also possess superior agronomic traits owing to the poor agronomic quality of the resistant parents. Thus, breeders have hoped to find more efficient selection methods based on the use of DNA markers. Genetic studies of resistance have progressed well[22-24], and elite CCW-resistant lines have been developed by means of marker-assisted selection combined with recurrent backcrossing[24].

The soybean resistance to insects is also important in the United States, where several lepidopteran insects

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have been recognized as serious pests of soybean[4]. The search for resistant germplasms and investigation of their resistance mechanisms preceded such studies in Japan. Although transgenic crops are generally accepted in the United States, the effects of the transgene alone is not always sufficient for effective pest management[33,34,48]. Because these researchers believed that very high production of the *Bt* protein was needed to prevent the development of *Bt*-resistant insects, the native resistance of soybeans is also being used in breeding programs as a means of preventing the development of *Bt* resistance[51,52].

Genetic resources for resistance to herbivorous insects

To develop cultivars resistant to the CCW or other lepidopteran insects by means of ordinary cross-breeding methods, the identification of a resistance donor is important as a first step. The search for such resistant germplasms began in the 1960s in the United States. Initially, three plant introduction (PI) lines from Japan (PI229358, PI227687 and PI171451) were shown to exhibit resistance to the Mexican bean beetle [*Epilachna varivestis* (Mulsant)][50]. These lines were confirmed to have resistance to other herbivorous insects. Hatchett et al.[13] reported that the three germplasms exhibited resistance to the corn earworm [*Helicoverpa* (*Heliothis*) *zea* (Boddie)] and the tobacco budworm [*Helicoverpa* (*Heliothis*) *virescens* (Fabricius)]. The three lines were also confirmed to resist the velvetbean caterpillar [*Anticarsia gemmatalis* (Hübner)], the soybean looper [*Pseudoplusia includens* (Walker)], and the beet armyworm [*Spodoptera exigua* (Hübner)][30]. Based on this research, the three lines were used as insect resistance donors in breeding programs, and some commercial cultivars were subsequently developed and released[7]. The search for resistant germplasms continued and other resistant lines were detected. Beach et al.[3] reported an introduced line (PI423968) that was resistant to the soybean looper. Kraemer et al.[25,26] detected more than 30 introduced soybean lines that exhibited significantly less defoliation by the Mexican bean beetle, although their resistance was no better than that in the first three PI lines. Among the new lines, some relatively early-maturing germplasms (PI416937 and PI416925) were used in breeding programs, and some of the progenies exhibited significant resistance to the corn earworm[35]. Rowan et al.[46] also detected breeding lines that exhibited moderate resistance to the corn earworm, velvetbean caterpillar, soybean looper, and beet armyworm. Some time later, Kraemer et al.[27] and Kraemer[28] found additional lines with resistance to the corn earworm. The resistance of these germplasms, especially that of the first three PIs, was analyzed and has been used in breeding programs in the United States.

When the breeding program to develop CCW-resistant soybean in Japan started in 1977, some genetic resources resistant to some lepidopteran herbivorous insects had been reported in the United States[50]. The screening of CCW-resistant germplasms in Japan took advantage of these preceding studies. The three PIs (PI229358, PI227687 and PI171451) ascertained to have insect resistance were confirmed to be resistant to CCW, too. The line named IAC-100 that was reported by Kraemer et al.[27] was also identified with the resistance. In addition, the resistant line 'Himeshirazu' was detected during the early research[12]. It exhibited resistance to the CCW that was similar to and sometimes higher than those of the three initial PIs[22]. Based on these resistant genetic resources, a breeding program for CCW resistance started.

Development of methods for evaluating insect resistance, and morphological or physiological studies of resistance

Since the detection of germplasms resistant to herbivorous insects, significant research results about the resistance of soybean have been achieved in the United States. A noteworthy aspect of this research has been an improvement of the methods for evaluating insect resistance. In general, there are three modes of plant resistance to insects: antibiosis, antixenosis (non-preference for the resistant plant), and tolerance[14]. Thus, researchers have developed procedures to evaluate each mode of insect resistance, especially for antibiosis and antixenosis. To evaluate antibiosis, researchers generally measure larval or pupal weights of insects reared on sample leaves[31], but some have instead measured pupal weights and the period to pupation[29] or the growth rate[45]. To evaluate antixenosis, researchers generally measure the degree of defoliation by the insect and compared that with a susceptible control[1,5].

In these attempts to evaluate resistance, morphological and physiological analyses have been conducted. As an example of the morphological approach, researchers have focused on the relationship between resistance and the degree of pubescence of soybean plants. Lambert et al.[31] reported that density of pubescence was related to the degree of antibiosis against several lepidopteran species. Kanno[18] confirmed that dense soybean pubescence increased antixenosis against the false melon beetle (*Atrachya menetriesi* Faldermann) compared with glabrous varieties. Other researchers have studied the relationship between tip shape of pubescence and the resistance. Hulburt et al.[15] reported that sharp tip pubescence increased both antixenosis and antibiosis. Despite these results, it may be unreasonable to conclude that pubescence is the

main cause of insect resistance. If dense pubescence is a main factor in resistance, most germplasms with dense pubescence should exhibit resistance. However, stating it empirically, many densely pubescent lines do not exhibit high resistance. Similar problems have been reported for pubescence shape. The location of the locus for pubescence tip shape (*Pb*) on a linkage map and those of the main quantitative trait loci (QTL) for insect resistance differ from each other[15,23,42-44]. Of course, both pubescence density and tip shape appear to have some effect on resistance, but the main factors responsible for resistance still remain unknown.

Researchers who are using the physiological approach to resistance analysis have examined the relationships between plant age or leaf position and insect resistance. Reynolds & Smith[45] have reported that antibiosis is lower for newly expanded leaves than for mature leaves. Nault et al.[40] reported that the antibiosis that originated in PI229358 was significant during the vegetative stage but not during the reproductive stage. Nault et al.[41] also reported that antixenosis varied during plant development. From these results, it is possible to establish a hypothesis that the concentration or composition of some chemical substances related to the resistance varies with plant or leaf age. The results of grafting tests have suggested the existence of a translocatable factor related to antibiosis[19], though the actual substance has not yet been identified.

The antibiosis and antixenosis exhibited by the three original resistant PIs, IAC-100, and Himeshirazu were re-confirmed using CCW larvae in Japan[37,38], though here we can refer to the result of Himeshirazu only. Antibiosis was evaluated using the pupal weight and duration from hatching to pupation of CCW reared on detached soybean leaves[39]. Antixenosis was evaluated by comparing the leaf area consumed in a susceptible line versus an evaluated line when CCW larvae were free to choose between the leaves in a petri dish[37]. Japanese researchers also reported that the pubescence of Himeshirazu and PI229358 was not a main factor in their antixenosis to CCW using glabrous near-isogenic lines[11]. Breeding programs to develop CCW-resistant soybean lines progressed based on these results.

In addition, Komatsu et al.[22] developed a new evaluation method for antibiosis resistance that was more suitable for use along with genetic analysis by reference from preceding studies, because previous methods required long periods and a large number of sample leaves to conduct an evaluation. In the research[22], the antibiosis of Himeshirazu and PI229358 against the CCW is confirmed again.

Because the new method has played a vital role in subsequent studies of CCW resistance, we have provided a detailed explanation here. The new bioassay uses sixth-instar CCW larvae that had been reared on artificial diet until the end of the fifth instar. A leaflet of the individual plant or line being evaluated was supplied to molted larvae every day until pupation. The pupal weight and duration to pupation (eight hours as a unit time) are both used to calculate an index of antibiosis (discussed below). Using this method, Komatsu et al.[22] detected a significant difference in pupal weight and the duration of the sixth instar among Himeshirazu, PI229358, and the CCW-susceptible cultivar 'Fukuyutaka' (Table 1). Differences in pupal weight and duration of the sixth instar were also detected between male and female larvae reared on Fukuyutaka. In male larvae, the pupal weight was light but duration of sixth instar was short compared with the female. These differences might be due to different growth patterns of male and female larvae[15], thus it is difficult to use only the pupal weight or the duration of the sixth instar as an index of antibiosis. To correct for the difference between sexes, the standardized insect-growth index (SII) was developed[22]. SII is an indicator of growth rate that equals the pupal weight divided by the sixth-instar duration. A higher SII means that the plant used to rear the larvae has lower antibiosis. The SII values of the three soybean lines differed significantly, but there was no significant difference between male and female larvae (Table 1), suggesting that the index corrects a bias of difference between the sexes. The SII evaluation method has been confirmed to save the time for evaluation and labor for rearing insects compared to previous methods that used newly hatched larvae. Thus, this method has been adopted in subsequent genetic studies of CCW resistance.

Genetic studies of resistance

In early times, the genetic aspects of the insect resistance were analyzed statistically using segregating populations about the resistance. Sisson et al.[47] reported that two or three genes were involved in the control of antixenosis of PI229358 against the Mexican bean beetle. Kilen & Lambert[21] determined that each of the three original insect-resistant PIs (PI229358, PI227687 and PI171451) had at least one gene that differed from the other lines. Kenty et al.[19] reported that the broad-sense heritability of PI229358-derived antixenosis against the soybean looper was 63%. Komatsu et al.[22] estimated that the broad-sense heritability of antibiosis of Himeshirazu against the CCW was 73.2%.

Since the late 1990s, when molecular genetic analysis became possible in soybean studies, antixenosis and antibiosis were investigated genetically. First, the antixenosis of PI229358 against the corn earworm was analyzed, and three QTLs were detected[42]. Shortly afterwards, the an-

Table 1. Antibiosis effects of soybean varieties for larval growth of the common cutworm evaluated using the standardized insect-growth index (SII)[22]

Germplasms	Number of larvae		Pupal weight (mg)		Duration of the sixth instar (8 hours as a unit time)		SII		
	♀	♂	♀	♂	♀	♂	♀	♂	weighted mean of both sexes
Fukuyutaka	40	44	349.6 a**	308.3 a**	18.09 a**	16.11 a**	19.44 a	19.30 a	19.39 a
PI229358	43	41	259.6 b	263.8 b	20.07 b*	19.08 b*	13.17 b	13.96 b	13.57 b
Himeshirazu	42	41	225.9 c	242.9 c	20.37 b	20.19 b	11.30 c	12.26 c	11.76 c

Soybean leaves for analysis were collected from V14-15, R1 stage[10] in Fukuyutaka, and V14-16 stage[10] in PI229358 and Himeshirazu. Values within a column followed by different letters differ significantly ($P < 0.05$, Tukey-Kramer multiple-comparison test).
**, *: Significant difference at $P < 0.01$ and $P < 0.05$, respectively, between female and male larvae (t-test).
SII: pupal weight / duration of the sixth instar.

tixenosis of PI227687 and PI 171358 against the corn earworm was also analyzed, and four QTLs were revealed[43]. The antibiosis of the three PIs against corn earworm was also analysed by Rector et al.[44], who detected a total of five QTLs. Terry et al.[49] detected nine QTLs for antibiosis resistance using two recombinant inbred lines derived from a cross between insect-susceptible parents.

In Japan, Komatsu et al.[23] performed a QTL analysis to identify the location and effects of the gene or genes involved in the antibiosis of Himeshirazu. A segregating population of 143 F_2 plants derived from a cross between Himeshirazu and Fukuyutaka was used in the study. Antibiosis of each plant was evaluated with six CCW larvae reared on each line and their mean SII value was calculated. A genetic map was constructed based on 146 simple sequence repeat (SSR) loci and a phenotypic locus (the *T* locus, which governs pubescence color). The resulting map consisted of 23 linkage groups and spanned a total of 2,270 cM. By means of composite interval mapping method[54,55], two QTLs for the antibiosis were detected in the linkage group M (LG-M), which had been defined by Cregan et al.[8]. For the QTL detection, QTL Cartographer 1.16 software[2] was used and the threshold of logarithm of odds (LOD) score was set as 3.55 based on a permutation test[6] with 1,000 times permutations in the study. A QTL with a higher LOD score was named CCW-1, and another with a lower LOD score was named CCW-2 (Common Cut-Worm 1 and 2, respectively; Fig. 1). The additive effect of CCW-1 and CCW-2 were re-estimated as 0.96 and 1.24 respectively by QTL Cartographer 2.5[6]. The dominance effect of CCW-1 and CCW-2 were 1.04 and -0.23, respectively in a new estimation.

The QTLs that have been detected so far are summarized in Table 2. The results suggest that a QTL located in LG-M (around the A584V and Sat_258 loci) plays an important role in both antixenosis and antibiosis, although it has not been confirmed that all of the QTLs and resistance alleles are identical. It is interesting that the resistance allele for this QTL has been estimated to be recessive in antibiosis[23,44]. Because some recessive genes that control resistance to insects have been reported in plants, these genes generally participate in the metabolism of chemical substances that are harmful to insects. For example, a gene for *Trichoplusia ni* resistance in *Arabidopsis thaliana* encodes a mutant form of an epithiospecifier protein; the wild-type promotes the hydrolysis of glucosinolate to nitrile[32]. Loss of function of the gene leads to the formation of isothiocyanate, which deters herbivores. In *Zea mays*, a recessive allele of a QTL for corn earworm resistance increases the concentration of maysin, which is a kind of C-glycosyl flavone that participates in antibiosis against the corn earworm[34]. It is also possible that some substances in soybean lines resistant to insects provide a similar mechanism for insect resistance.

Another QTL in LG-M (around the Satt567 locus) is also worthy of note in regard to the genetic control of the insects resistance. Komatsu et al.[23] and Terry et al.[49] have detected a QTL near that locus, but reported different effects for the QTL. The effect of the QTL from Minsoy is lower than that of the gene from Himeshirazu (i.e., has a lower R^2 value). Besides, Minsoy is not an insect resistant cultivar. It is possible that the two loci are identical but that Minsoy and Himeshirazu have different alleles at this locus. Such a situation (i.e., many alleles at a locus

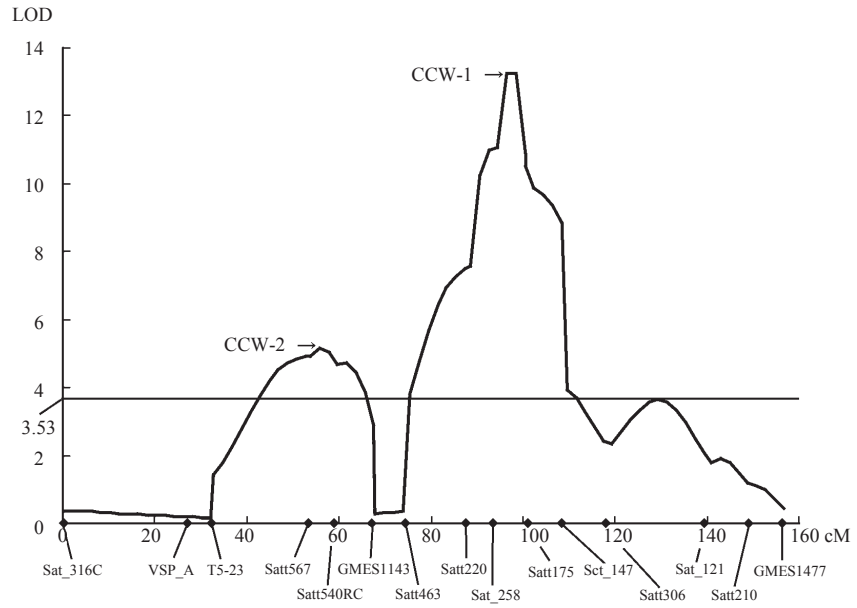

Fig. 1. Logarithm of odds (LOD) scores associated with the standardized insect-growth index (SII) in linkage group M

In this figure, the LOD scores were estimated by means of composite interval mapping in QTL Cartographer 2.5[2]. An LOD score of 3.53 was used for the QTL detection threshold based on a Type I error rate of 5%. All of the DNA markers were simple sequence repeats. GMES1143, GMES1477, VSP_A, T5-23, Sat_316C, and Satt540RC were developed from EST or genome sequences. The others are published in the USDA soybean genetics and molecular biology database (http://soybase.org/).

that differ in their effect) agrees with the observation that insect resistance in soybean exhibits continuous variation among germplasms[27,28].

A QTL for antixenosis (around the R249T) located in linkage group H (LG-H) was detected in the three insect-resistant PIs[42,43]. This QTL has not been detected in any genetic analysis of antibiosis. This suggests that different mechanisms may act in antixenosis and antibiosis. If the different mechanisms are controlled by different genes, QTL location would vary. In any event, the utilization of many genes or mechanisms will be important in breeding programs to prevent the breakdown of resistance, and the QTL in LG-H will be valuable even though its effect was lower than that of the QTL in LG-M.

It is remarkable that susceptible germplasms have many resistance alleles. In particular, an allele for the QTL in linkage group F from insect susceptible "Cobb" has a stronger effect (R^2 = 20 to 33%) than many other resistance alleles (Table 2). Although the analysis and application in breeding programs of these QTLs has not yet progressed very far, it is clear that they will potentially play an important role in the development of high-resistance cultivars by means of gene pyramiding.

Validation of insect resistance QTLs

A number of QTLs for insect resistance have been detected by means of genetic analysis, and it has thus become possible to develop insect-resistant cultivars using QTLs and their genetic information. However, it is necessary to verify the effectiveness of each QTLs before using them in actual breeding programs, because these QTLs have been predicted by statistical methods of QTL analysis not based on the results of field trials. Thus, some researchers have attempted to confirm the effectiveness of the QTLs detected from the three insect-resistant PIs and Himeshirazu.

The effect of a QTL located in LG-M (around A584V and Sat_258 marker loci) that was detected in PI229358, PI171451, and Himeshirazu has been verified for antixenosis and antibiosis. Narvel et al.[39] found that PI229358 and PI171451 alleles were conserved in most insect-resistant lines developed by traditional cross-breeding. Walker et al.[51,52] confirmed the effect of the QTL in antixenosis and antibiosis using near-isogenic lines derived from PI229358. Zhu et al.[56] also confirmed the effect of a PI229358 allele, and located the gene between Sat_258 and Satt702, which represents a span of 0.52 cM. Komatsu et al.[24] reported

Table 2. QTLs for resistance of soybean against herbivorous pests

Origin of the resistance allele	Mode of resistance	Linkage group	Flanking marker	LOD [1)]	R^2 [2)]	References
PI229358	antixenosis	D1b+W	Bng047D	2.0	10	Rector et al.[42] (1998)
		H	R249T	4.0	16	Rector et al.[42] (1998)
		M	A584V	10.1	37	Rector et al.[42] (1998)
	antibiosis	G	L002H	3.8	19	Rector et al.[44] (2000)
		M	A584V	4.8	22	Rector et al.[44] (2000)
PI227687	antixenosis	C2	A132T-1	2.2	11	Rector et al.[43] (1999)
		H	R249T	1.8	9	Rector et al.[43] (1999)
	antibiosis	B2	A343V_2	2.2	12	Rector et al.[44] (2000)
PI171451	antixenosis	H	R249T	3.7	19	Rector et al.[43] (1999)
		M	A584V	9.7	37	Rector et al.[43] (1999)
	antibiosis	M	A584V	5.0	28	Rector et al.[44] (2000)
Himeshirazu	antibiosis	M	Sat_258	13.2	24	Komatsu et al.[23] (2005)
		M	Satt567	5.1	9	Komatsu et al.[23] (2005)
Cobb	antixenosis	F	B212V_2	4.8	20	Rector et al.[43] (1999)
	antibiosis	F	A0831	3.8	33	Rector et al.[44] (2000)
		J	K401H	2.8	19	Rector et al.[44] (2000)
Minsoy	antibiosis	C2	Satt365	-	7	Terry et al.[49] (2000)
		D1a+Q	R013_2	-	8	Terry et al.[49] (2000)
		E	Sat_121	-	17	Terry et al.[49] (2000)
		E	Sat_121	-	9	Terry et al.[49] (2000)
		E	Sat_121	-	12	Terry et al.[49] (2000)
		H	Satt302	-	8	Terry et al.[49] (2000)
		H	Satt192	-	6	Terry et al.[49] (2000)
		H	Satt302	-	9	Terry et al.[49] (2000)
		M	Satt567	-	7	Terry et al.[49] (2000)

1): Logarithm of Odds. For Minsoy, the exact LOD values were not published.
2): The proportion (%) of the total phenotypic variance explained by the locus.

that the QTL around the A584V and Sat_258 marker loci detected in PI229358 and Himeshirazu is the same locus. They also confirmed that both the PI229358 allele and Himeshirazu allele actually provide antibiosis to CCW (Table 3). In the studies in Table 3, near-isogenic lines (NILs) were developed for QTLs in LG-M derived from CCW-1, CCW-2 and PI229358 by recurrent backcrossing with Fukuyutaka. Additionally, F_1 hybrids of Fukuyutaka and an NIL for CCW-1, of Fukuyutaka and an NIL for a PI229358-derived QTL, and of an NIL for CCW-1 and an NIL for a PI229358-derived QTL have also been developed. All of the NILs exhibited significantly lower SII values than those of the recurrent parent Fukuyutaka, though there was some inconsistency between experiments. The F_1 plants of Fukuyutaka and an NIL for the CCW-1 or PI QTLs exhibited SII values similar to those of Fukuyutaka. This indicates that the resistance alleles are recessive as previously estimated in the QTL analysis. On the other hand, the F_1 plants for the cross between an NIL for CCW-1 and an NIL for PI229358 exhibited lower SII values than those of Fukuyutaka or other F_1 hybrids. If the CCW-1 and PI229358 loci differ, then the F_1 plants

Table 3. Genotypes at the CCW-1 and CCW-2 loci, and standardized insect-growth index (SII) values for near-isogenic lines developed by means of marker-assisted backcrossing

Lines	Genotype [1]		SII (mean ± S.D.) [2]
	CCW-1	CCW-2	
Himeshirazu	Hime	Hime	9.68 ± 0.87 a
Kyushu 155	Hime	Hime	11.33 ± 0.95 b
Kyuko 1204	Fuku	Hime	14.36 ± 1.03 c
Kyukei 356	Hime	Fuku	15.40 ± 1.31 c
Fukuyutaka	Fuku	Fuku	17.05 ± 0.80 d
PI229358	PI	PI	9.47 ± 0.85 a
Kyukei 356/ Kyuko 1206	Hime/PI	Fuku	16.35 ± 0.94 b
Kyuko 1206	PI	Fuku	17.11 ± 0.98 b
Fukuyutaka/ Kyukei 356	Fuku/Hime	Fuku	19.20 ± 0.87 c
Fukuyutaka/ Kyuko 1206	Fuku/PI	Fuku	20.05 ± 1.21 c
Fukuyutaka	Fuku	Fuku	20.51 ± 0.92 c

1): Hime, Fuku and PI indicate that the locus is fixed for the Himeshirazu, Fukuyutaka and PI229358 alleles, respectively.

2): Values followed by different letters differ significantly ($P < 0.05$, Tukey-Kramer multiple-comparison test). Because of differences in the experimental conditions, the SIIs in the upper and lower sections of the table should not be compared directly. Upper section is original data and lower section refers to Komatsu et al.[24].

of the NILs should exhibit SII values similar to those of Fukuyutaka since their resistance alleles are recessive. Thus, the two QTLs appear to be identical.

The effect of another QTL (around Satt567) in LG-M that has been detected in Himeshirazu and named CCW-2 is also evident (Table 3). Komatsu et al.[24] confirmed that this Himeshirazu allele actually suppressed the growth of CCW larvae and the effect equaled that of the other resistance gene in LG-M (around Sat_258). In addition, no interaction was detected between the resistance alleles of the QTL. We think that the efficiency of lines that only possess the resistance gene in LG-M around Sat_258 is not entirely trusted in breeding programs, thus the additional gene around Satt567 should be important.

On the other hand, the effect of QTLs in linkage groups D1b+W (LG-D1b+W), G (LG-G), and H have not yet been thoroughly investigated. Narvel et al.[39] estimate that the effects of the QTLs in LG-D1b+W and LG-H are either very limited or do not exist. Walker et al.[52] also detected no antixenosis effect of the QTL in LG-H, and Warrington et al.[53] detected little influence of the resistance alleles in the QTLs in LG-G and LG-H on antixenosis and antibiosis. Although these reports cast doubt upon the effects of these QTLs, Zhu et al.[56] detected a genetic interaction between the QTL resistance alleles in LG-M and those in LG-G or LG-H. They reported that the QTL resistance allele in LG-G or LG-H has no effect by itself, but exhibits an effect when the QTL in LG-M is fixed for the resistance allele. This phenomenon could explain the previous failures to confirm the effects of these QTLs, but contradictory results have been reported by the same authors[57]. In the latter study, they detected a slight antibiosis effect from the QTL in LG-G alone but found no significant interaction between the QTLs in LG-M and LG-G. These results suggest that the QTLs in LG-D1b+W, LG-G and LG-H should not be expected to have effects by themselves in breeding programs.

A new direction for studies of and breeding for insect resistance in soybean

Despite a long period of research, CCW-resistant soybean cultivars acceptable to farmers have not yet been released in Japan, even though resistant germplasms have been detected and analyzed on the genetic features. In the United States, insect resistance breeding would be in a similar state if transgenic breeding methods had not been used. The main cause of the difficulty encountered by Japanese breeding programs has been the poor agronomic traits of the insect-resistant parents. The progeny of crosses between resistant donor parents and other soybean lines necessarily led to the inheritance of some agriculturally undesirable traits.

Recurrent backcrossing using DNA markers as selection indicators is an effective solution for this problem because it enables breeders to reduce the presence of the genome region of resistant parents that relates to the poor agronomic traits. In Japan, a line (Kyushu 155) recently developed by marker-assisted recurrent backcrossing combines the resistance to the CCW and excellent agronomic characteristics[24]. The line inherits two resistance genes from Himeshirazu (Table 3), but the maturity, plant height, number of branches, yield, and seed qualities are similar to those of the recurrent parent Fukuyutaka (data not shown). Kyushu 155 is now being distributed to agricultural experiment stations for evaluation of adaptability for cultivation. In the United States, some lines with insect resistance and desirable agronomic traits have been developed as commercial cultivars[53].

Thus, it is possible to predict that soybean breeding for insect resistance is entering a new phase. Hereafter, the efficiency of the resistance genes in the field should be evaluated from an ecological perspective. For exam-

ple, Kyushu 155 exhibits resistance to the CCW, but it is not yet known how many insecticide applications will be needed to provide effective CCW management in the field. For this cultivar to be widely adopted by farmers, some criterion or standard for insecticide application based on firm scientific evidence must be provided. In addition, ecological studies on the relationship between the cultivation of resistant cultivars and development of ability to assault the resistant cultivar in insects will be essential to prevent breakdown of the resistance. A comprehensive model of insect resistance and insecticide application that will prevent the breakdown of resistance is not yet available. Thus, a model to prevent the breakdown of the insect resistance and to maximize the economic and ecological benefits from the reduction of insecticide applications should be developed based on ecological investigations.

In addition to the ecological studies, further research is required to identify the direct causes and mechanisms of resistance. This is necessary because identifying the mechanism of resistance will help researchers to identify new resistant germplasms, and prevent the breakdown of resistance by combining different resistance genes. We believe that the key to progress in these investigations will lie in more complete genome information for soybean. The U.S. Department of Energy Joint Genome Institute has released preliminary whole-genome information for soybean (http://www.phytozome.net/soybean.php). This information will make it easier to develop new DNA marker loci in interesting regions of the soybean genome. Close markers with interesting genes enable us to identify the genes through map-based cloning methods[17]. Although in soybean such a genetic approach has rarely been used until recently, it will become an increasingly important strategy in the future.

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