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Stress, Physiological and Genetic Factors of Rice Leaf Bronzing in Paddy Fields

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ABSTRACT

Leaf bronzing, a nutrient disorder in paddy fields, can strongly depress rice growth and grain yields in severe cases. Studies using hydroponic or pot culture have clarified its cause as nutritional stress, e.g., Fe^{2+} or Mn^{2+} excess, and Zn^{2+} deficiency. According to complex genetic factors, rice cultivars widely exhibit various tolerances to nutritional stress: The rank order of cultivars' leaf bronzing shown in excess Fe^{2+} hydroponic cultures does not necessarily correspond to that of cultivars in Fe^{2+} excess fields. Furthermore, leaf bronzing does not occur in excessively saline hydroponic culture, but brown spots occur on leaf blades of some lines in saline-flooded paddy fields. Therefore, leaf bronzing occurring in fields might be induced by other stresses and genetic factors aside from stress in hydroponic or pot culture. These facts indicate that studies of hydroponic and pot cultures are insufficient to elucidate physiological and genetic factors. This report reviews findings associated with physiological and genetic diversity for leaf bronzing in paddy fields, hydroponic cultures, and pot cultures.

Keywords: genetic diversity, leaf bronzing, nutritional stress, paddy field, quantitative trait loci, rice
Abbreviations: CSSL, chromosome segments substitution line; **NIL**, near isogenic line; **QTL**, quantitative trait loci

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INTRODUCTION

Visible symptoms that occur on rice leaf blades provide an important signal of the start or increase of the influences of stress factors such as nutritional stress, ultraviolet-B radiation, blast fungus, and virus. Brown spots on leaf blades caused by nutritional stress have been called by various names such as Bronzing and Akagare types I, II and III. For this review, we designate these brown spots generally as leaf bronzing. Leaf bronzing is widely observed throughout wet zones of Asian paddy fields (Ponnamperuma *et al*. 1955; Tajima and Baba 1962; Ota 1968). Leaf bronzing inhibits the growth of shoot length, tiller number and biomass (Ponnamperuma *et al*. 1955; Ota 1968). Consequently, it decreases the grain yield of rice remarkably.

Rice is the major staple food for half of the world's population. For that reason, numerous studies have investigated nutritional stress factors causing leaf bronzing of rice in paddy fields, from the 1950s. Many researchers have sought to identify stress factors through classification of leaf bronzing using observations on their occurrence at difference growth stages, affected tissues, and colors of symptoms (Ponnamperuma *et al*. 1955; Tajima and Baba 1962; Ota 1968; Tensho and Yeh 1970). Those studies described leaf bronzing as related to various nutritional stresses. However, the studies did not identify the specific stresses or the physiological and genetic factors of leaf bronzing that occurs in paddy fields.

Many genetic factors affecting susceptibility to leaf bronzing have been identified in the rice genome. That knowledge has been used to improve genetic diversity, to develop tolerant cultivars, and to clarify tolerance mechanisms for nutritional stress (Wu *et al*. 1997; Wang *et al*. 2002; Wan *et al*. 2003; Dong *et al*. 2006; Takehisa *et al*. 2006; Wissuwa *et al*. 2006). Herein, we mainly describe the genetic diversity related to leaf bronzing by the following points: 1) stress factors causing leaf bronzing in paddy soils, 2) physiological factors related to leaf bronzing, 3) genetic factors affecting leaf bronzing. Additionally, we specifically address the differences and inconsistencies of genetic diversity under paddy field, hydroponic, and pot cultures. Finally, we discuss a strategy to identify the factors related to stress-related, physiology, and genetic background and to develop nutritional-stress tolerant rice cultivars for growth in paddy fields.

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Invited Mini-Review

STRESS FACTORS

The stress factors of leaf bronzing in rice grown in peaty, boggy soil or acidic diluvial soil paddy field with excess humus in Japan have been studied (Baba *et al.* 1954; Baba and Tajima 1961; Tajima and Baba 1962). Initially, leaf bronzing was classified according to symptoms such as the growth stage, affected plant part, and appearance. Baba *et al.* (1954) described the first sign of the symptom: its appearance at the leaf-tips. In contrast, Baba and Tajima (1961) observed the symptom of leaf chlorosis before the appearance of reddish brown discoloration of the leaf blade in peaty and boggy soil paddy fields. Moreover, Tajima and Baba (1962) identified symptoms that appeared as small brown spots on leaves and the appearance of dead and blackened tissues in the lower internodes of stems in paddy fields of acidic diluvial soil, which had been converted from upland fields or which had been newly reclaimed. These instances of leaf bronzing were distinguished respectively by their phenotypes, and were named Akagare types I, II and III (Baba and Tajima 1961; Tajima and Baba 1962).

Next, the stress factors of each type of leaf bronzing were studied through analyses of the paddy soil component, or the nutritional concentration in plants grown under hydroponic or pot culture. Results showed that the main factor of Akagare type I was the presumed potassium deficiency in plants because the K concentration was lower in leaf bronzing plants; furthermore, added K reduced leaf bronzing (Tajima and Baba 1962; Baba *et al.* 1955). For Akagare type II, it was presumed by soil component analyses that the main factor was absorption of excess Fe^{2+} and H_2S, which were produced in reduced soil, and hydroponic culture with Fe^{2+} or low pH (Baba and Tajima 1961). In contrast, Tanaka (1995) reported that the main factor of Akagare type II was Zn^{2+} deficiency because the symptoms were promoted by Zn^{2+} deficiency and alleviated by adding Zn^{2+} in hydroponic and pot cultures with field soil. Tajima and Baba (1962) indicated that the main factor of Akagare type III was some unknown substance which differed from that causing Akagare types I and II because the occurrence of Akagare type III was correlated with K or Fe concentrations in plants; it was not always promoted merely by reduced soil. Tensho and Yeh (1970) indicated that the main factor of Akagare type III was iodine-ion (I^-) toxicity because the symptoms were promoted in hydroponic cultures with I^- toxicity.

In Sri Lanka, leaf bronzing is called simply Bronzing; it appears on older leaves of rice grown in submerged soil and paddy fields (Ponnamperuma *et al.* 1955). Ponnamperuma *et al.* (1955) observed that the tips of these leaves became reddish-brown; the coloration spreads toward the base, especially along the edges. They reported that the main factor of Bronzing was Fe^{2+} toxicity because lime inhibits the buildup of a high concentration of Fe^{2+} in soil solution. On the other hand, Ota and Yamada (1962) reported that Bronzing was promoted by Al toxicity under the condition of Ca^{2+} deficiency using hydroponic culture. They presumed that the main factor of Bronzing was accumulation of excess Al, along with Ca deficiency in plants because the occurrence of Bronzing respectively showed positive and negative correlation with Al and Ca concentrations in plants. Inada (1964) reported that Bronzing was promoted by the coexistence of excess Fe^{2+} and H_2S in hydroponic culture. Moreover, the Bronzing leaves reportedly showed higher Fe contents and lower Mn contents than healthy leaves. Therefore, Inada inferred that Bronzing was promoted by inhibition of physiological activity of roots by toxic substances such as Fe^{2+} and H_2S. Consequently, excessive absorption and accumulation in plant of Fe^{2+} takes place while inhibiting absorption of nutrients such as Mn^{2+}.These results from investigations in Japan and Sri Lanka suggest that leaf bronzing is related to various stresses: Fe^{2+} and H_2S toxicity, and deficient K^+, Zn^{2+} and Mn^{2+}.

Excess ferrous Fe^{2+} dissolves into the soil solution at ill-drained paddy fields (Suzuki *et al.* 1999). The oxidation-reduction potential (ORP) decreases in plowed soils because gaseous oxygen is not supplied when a paddy field is kept under swamp conditions (Kohno *et al.* 1995). When oxygen is used up, Fe, Mn, and sulfate are reduced; consequently, Fe^{2+}, Mn^{2+} and H_2S are produced in soil solution (Kohno *et al.* 1995). Consequently, excess Fe^{2+}, Mn^{2+} and H_2S toxicities occur in flooded paddy fields, as in ill-drained paddy fields. Moreover, nutrient toxicity and deficiency in rice often occur to varying degrees in acidic soil or alkaline soil paddy fields. In acidic soils, excess Fe^{2+}, Mn^{2+}, Al^{3+}, and Zn^{2+} toxicities, in addition to K^+ deficiency often inhibit rice growth, as do Zn^{2+}, Fe^{2+} and Mn^{2+} deficiencies in alkaline soil (Neue *et al.* 1998). These results indicate that varied nutritional stresses intricately affect rice growth in ill-drained paddy fields. Multiple nutritional stresses, not individual stresses, cause leaf bronzing. Therefore, it is difficult to identify stress factors of leaf bronzing in a paddy field solely using visual evaluation, paddy soil component analysis, or nutritional concentration in plants.

GENETIC DIVERSITY AND PHYSIOLOGICAL MECHANISM

Genetic diversity of leaf bronzing was observed in rice grown in paddy fields and soil pots with various nutritional stresses (Baba *et al.* 1956; Baba and Tajima 1961; Tajima and Baba 1962). Tajima and Baba (1962) reported that the tolerant and sensitive cultivars to Akagare type III almost all showed tolerance and sensitivity to Akagare type I and II. Moreover, the native cultivars of wet-zone paddy fields showed greater tolerance for bronzing than the dry zone cultivars (Ota 1968). These results suggest that rice genotypes differ widely in their tolerance for leaf bronzing related to nutritional stresses, and present the possibility of development of rice cultivars with enhanced tolerance through breeding programs. The characterization of genetic diversity could be anticipated as a first step to elucidate the tolerance mechanisms of leaf bronzing that are affected by various stresses in paddy field cultivation.

Leaf bronzing occurs by several nutritional stresses (above section). Therefore, to clarify the tolerance mechanism of leaf bronzing, it is valuable to identify the other physiological traits, which correlated with occurrence of leaf bronzing in rice grown under hydroponic cultures with several nutritional stress, such as Fe^{2+} and Mn^{2+} toxicity, and Zn^{2+} deficiency. The physiological traits correlated with avoidance and tolerance mechanisms of rice for these nutritional stress, have been described in some reviews and reports, in detail (Dobermann and Fairhurst 2000; Sahrawat 2004; Becker and Asch 2005; Hoffland *et al.* 2006). In this review, we focused on the tolerance mechanism related to the genetic diversity of leaf bronzing among them, and discussed it.

Regarding Fe^{2+} toxicity, Sahrawat (2004) introduced that three functions of rice roots were involved in counteracting the toxicity: 1) Oxidation of Fe in the rhizosphere, which helps to keep Fe concentration low in the growth media; 2) Iron-excluding power of the roots, which excludes Fe at the root surface and thus prevents Fe from entering the root; 3) Iron retaining power of the roots, which retains Fe in the root tissue and thus decreases the translocation of Fe from the root to the shoot. Becker and Asch (2005) divided the functions into two mechanisms as avoidance and tolerance mechanisms. An avoidance mechanism included the three root functions, and indicated the mechanism of excluding Fe^{2+} at the root level, thereby avoiding Fe^{2+} damage to the shoot tissues through root ion selectivity and excluding Fe uptake. In contrast, the tolerance mechanism indicated the mechanism using elevated levels of Fe^{2+} within leaf cells, probably *via* enzymatic detoxification of oxygen species in the symplast (Becker and Asch 2005). Large amounts of Fe in plants could give rise to the formation oxygen radicals, which are phytotoxic and responsible for protein degradation and peroxidation on membrane lipids (Dobermann and Fairhurst 2000). Excessive Fe uptake also results in increased polyphenol oxidase activity (Dobermann and Fairhurst 2000). The activity of phenol oxidases increases, and oxidized polyphenols accumulate causing leaf bronzing symptoms (Peng and Yamauchi 1993).

The avoidance ability in rice could be presented by measuring of the Fe concentration in plants and oxidation power of roots (Ottow *et al*. 1982). On the other hands, Yamauchi and Peng (1995) found no correlation between the Fe concentration and leaf bronzing of any rice genotype. Furthermore, Luo *et al*. (1997) indicated that leaf bronzing-tolerant cultivars had higher N, P, K, and Mg concentrations in plants than sensitive cultivars in hydroponic cultures, but no significant promotion of leaf bronzing was detected with increasing Fe concentration using 10 cultivars. Therefore, these results indicate that the varietal difference for leaf bronzing is not always related to "avoidance mechanisms" indicated by Becker and Asch (2005). Detoxification enzyme (peroxidase, ascorbate peroxidase, glutathione reductase, and dehydroascorbate reductase) activities in leaf-bronzing-tolerant rice cultivars were higher than those of sensitive cultivars in hydroponic cultures with Fe^{2+} toxicity (Bode *et al*. 1995; Wu *et al*. 1998). Superoxide dismutase and peroxidase activities in roots of cultivar IR97 were about twice those of a sensitive cultivar: IR64 (Bode *et al*. 1995). Especially, the activity of peroxidase in IR97 increased with higher iron concentration in hydroponic cultures, although the phenomenon was not so pronounced for IR64. Moreover, ascorbate peroxidase, glutathione reductase, and dehydroascorbate reductase activities of a tolerant cultivar, 'Azucena', were higher than those of a sensitive cultivar, 'IR64', under Fe^{2+} stress (Wu *et al*. 1998). Peng and Yamauchi (1993) indicated that the accelerated peroxidase activity was associated with bronzing development. Therefore, the genetic diversity of leaf bronzing might be more closely related to the tolerance mechanism *via* enzymatic detoxification than the avoidance mechanism, as Fe^{2+} exclusion under Fe^{2+} toxicity. However, Asch *et al*. (2005) reported that the correlations of between tissue Fe concentration and leaf bronzing in young seedlings (14 days after sowing in Fe^{2+} stress) was not significant, but in old seedlings (28 days after sowing in Fe^{2+} stress) was significant, using 14 lowland-rice genotypes. The result suggested that the genetic diversity about physiological mechanism of leaf bronzing under Fe^{2+} stress might not always unrelated to the avoidance mechanisms.

Regarding the mechanism of Mn^{2+} toxicity, rice has avoidance and tolerance mechanisms. Reportedly, the avoidance ability is the root oxidation power to oxidize Mn^{2+} in the rhizosphere, as with Fe^{2+} toxicity (Dobermann and Fairhurst 2000); the tolerance ability is indicated by the retention of Mn in root tissues (Dobermann and Fairhurst 2000). However, Nelson (1983) indicated that the tolerance to Mn^{2+} toxicity is unrelated to reduced uptake of the element. Moreover, results obtained using 150 lines derived from a cross between tolerant cultivar 'Azucena' and sensitive cultivar 'IR1552' indicate that no significant correlation exists between leaf bronzing and Mn contents or Mn concentrations in shoots (Wang *et al*. 2002). Therefore, the genetic diversity of leaf bronzing under Mn^{2+} toxicity might also be more closely related to the tolerance mechanism than to an avoidance mechanism, as with Fe^{2+} toxicity.

Hoffland *et al*. (2006) reported that the genetic diversity for tolerance of Zn^{2+} deficiency in rice is related to the absorption mechanism *via* exudation of higher amounts of citrate. However, it remains unclear whether the genetic diversity of leaf bronzing is related to the mechanism under Zn^{2+} deficiency. These results indicate that the genetic diversity of leaf bronzing by at least nutrient toxicity might be related more closely to the tolerance mechanism than to the avoidance mechanism. However, the tolerance mechanism and physiological factors of leaf bronzing also remain unclear.

GENETIC FACTORS

Based on the genetic diversity described above, many genetic factors of leaf bronzing have been identified through QTL analysis (**Table 1**), which is a linkage analysis using molecular markers and linkage maps. It is useful to detect multiple genetic factors for quantitative traits. Recently, molecular markers have facilitated the identification of chromosomal regions associated with many complex traits in rice using QTL analysis (Yano and Sasaki 1997). Wu *et al*. (1997) detected two QTLs for leaf bronzing on chromosome 1 using a doubled haploid (DH) population consisting of 123 lines derived from the tolerant cultivar 'Azucena' and the sensitive cultivar 'IR64' under hydroponic culture adding Fe^{2+} toxicity, in glasshouse. Moreover, Wan *et al*. (2003) identified a QTL on chromosome 1 using 96 backcross inbred lines (BILs) derived from a backcross of the tolerant cultivar 'Nipponbare' and the sensitive cultivar 'Kasalath' under hydroponic culture adding Fe^{2+} toxicity, in greenhouse. The two QTLs (*RG345*/*RG381* and *C955*/*C885*) identified by Wu *et al*. (1997) and Wan *et al*. (2003) are located in nearby regions on chromosome 1. These results suggest that two QTL are located on the same loci, and that they are important for leaf-bronzing tolerance of rice in terms of Fe^{2+} toxicity (Wan *et al*. 2003).

Regarding Mn^{2+} toxicity, Wang *et al*. (2002) identified five QTLs for leaf bronzing positioned near the centromere of chromosome 3, and on chromosomes 4, 5, 6 and 10, using the recombinant inbred line (RIL) population, consisting of 150 lines derived from a cross between the tolerant cultivar 'Azucena' and the sensitive cultivar 'IR1552' in soil pot adding Mn^{2+} toxicity, in greenhouse. Dong *et al*. (2006) identified three QTLs for leaf bronzing on chromosome 1, the short arm of chromosome 3, and chromosome 10 under a soil pot adding Zn toxicity outdoors. They used RIL population comprising 71 lines from a cross between tolerant cultivar 'Asominori' and a sensitive cultivar 'IR24'. Moreover, the QTL for Fe^{2+} toxicity (*RG810*/*RG331*) by Wu *et al*. (1997) and the QTL for Zn^{2+} toxicity (*XNpb93*/*C3029C*) by Dong *et al*. (2006) were identified in nearby regions on chromosome 1, irrespective of different populations and treatments (Wan *et al*. 2003). These results indicate that the QTLs might be related to the tolerance of leaf bronzing in Fe^{2+} toxicity and Zn^{2+} toxicity (Dong *et al*. 2006).

INCONSISTENCY OF GENETIC DIVERSITY OBSERVED IN PADDY FIELDS, HYDROPONICS AND POT CULTURES

Recently, Wissuwa *et al*. (2006) reported that the tolerance rankings obtained in low-Zn hydroponic culture did not concur with tolerance rankings in the Zn-deficient field using several RILs derived from the sensitive cultivar 'IR74' and the tolerant cultivar 'Jalmagna'. Moreover, the leaf bronzing was significantly correlated with Zn concentrations in shoots of rice grown in hydroponic culture, but not in the field. In other cases, it was reported that NILs showing Fe^{2+} tolerance were selected under hydroponic cultures with Fe^{2+} toxicity. However, these NILs' tolerance was not always observed in paddy fields with excess Fe^{2+} toxicity (Dr. Takuhito Nozoe, pers. comm.). Moreover, a Fe^{2+} tolerant line under hydroponic culture showed sensitivity in comparison to check variety in a paddy field (pers. comm.). These facts suggest that physiological and genetic factors of leaf bronzing in hydroponic or pot cultures do not accord completely with those in paddy fields. These inconsistencies seem to relate to the nature of tolerance needed under field conditions where soil factors are other causes of the nutrient problem. Gregorio *et al*. (2002) suggested that various mineral deficiencies, submergence, water depth, and drought, in addition to salt stress compound problems of salinity in paddy fields. Rice growth in saline soils depends on the interactions among soil factor, such as high levels of exchangeable sodium and micronutrient deficien-

Table 1 Mapping of QTL for leaf bronzing in rice grown in hydroponics, pot cultures and paddy fields.

Conditions	Parents cultivers (tolerance/sensitive)	Chromosome	Marker interval	LOD score[a]	References
In hydroponics or pot cultures					
Fe^{2+} toxicity	Nipponbare/Kasalath	1	*C955/C885*	3.2	Wan *et al.* 2003
	Azucena/IR64	1	*RG345/RG381*	10.1	Wu *et al.* 1997
		1	*RG810/RG331*	3.5	
Mn^{2+} toxicity	Azucena/IR1552	3	*RG227/AGG-CAG7*	2.5	Wang *et al.* 2002
		4	*RG163/AGG-CAG7*	6.1	
		5	*ACA-CTC/ACA-CAT3*	2.5	
		6	*RG213/RZ144*	2.8	
		10	*RZ500/AAC-CAG1*	5.1	
Zn^{2+} toxicity	Asominori/IR24	1	*XNpb93/C3029C*	6.0	Dong *et al.* 2006
		3	*R1468B/C515*	3.2	
		10	*C751B/C148*	2.2	
In paddy fields					
Zn^{2+} deficiency	Jalmagna/IR74	1	*RZ154-3/P1M10-14*	3.2-4.4	Wissuwa *et al.* 2006
(Wet and dry seasons)		1	*RG220/RG109*	4.1	
		4	*RG788/ P2M5-13*	2.6-4.7	
		7	*P2M7-7/P2M6-5*	2.7	
		12	*P3M5-9/P1M7-4*	3.6	
Salinity	Nipponbare/Kasalath	3	*R1925*	31.7	Takehisa *et al.* 2006
		11	*C1350/C477*	3.5	

[a] LOD indicate log odds

cies (Neue *et al.* 1998). Moreover, Sahrawat (2004) motioned that iron toxicity is a complex nutrient disorder and deficiencies of other plant nutrients, especially P, K, Ca, Mg and Zn, have been considered to affect its incidence in rice, in his review. Considering these information, we think that rice grown might be always compounded by several stresses, as mineral deficiencies, submergence, deep water, drought and salt stress in paddy fields. Therefore, it might be thought that the field conditions were often not simulated in pot or hydroponics cultures. For that reason, it is necessary to analyze the physiological and genetic factors of leaf bronzing in paddy fields directly to clarify the mechanisms of leaf bronzing tolerance in paddy fields.

CLARIFICATION OF STRESS, PHYSIOLOGICAL AND GENETIC FACTORS IN PADDY FIELDS

For the reasons mentioned above, it is very difficult to clarify the stress-related, physiological and genetic factors of rice in outdoor paddy fields using only visual evaluation or only analyses of physiological and genetic factors in hydroponic and pot cultures. Therefore, we suggest strategies of clarifying these factors of leaf bronzing using genetic diversity, which are shown as follows: 1) elucidation of physiological factors related to QTLs which were detected in paddy fields, using some hydroponic or pot culture assay systems, 2) identification and conformation of QTLs in paddy field and some hydroponic or pot culture by CSSL and NIL, 3) isolation of gene(s) controlling leaf bronzing in paddy fields.

First, it is necessary that QTLs for leaf bronzing be directly detected in paddy fields (ex. with various nutritional stresses) using mapping populations segregated for leaf bronzing. Wissuwa *et al.* (2006) detected five QTLs for leaf bronzing on chromosomes 1 (two), 4, 7, and 12 using a Zn-deficient paddy field, with an RIL population comprising 165 lines derived from the sensitive cultivar 'IR74' and the tolerant cultivar 'Jalmagna' (**Table 1**). We observed leaf bronzing of rice grown in a paddy field flooded with saline water and identified two QTLs controlling leaf bronzing using a BIL population consisting of 98 lines derived from 'Nipponbare' and 'Kasalath' cultivars (**Table 1**, Takehisa *et al.* 2006). These QTLs identified by Wissuwa *et al.* (2006) or Takehisa *et al.* (2006) is located in different regions from those of the QTLs identified under hydroponic and pot cultures with various nutritional stresses (**Table 1**). Therefore, these QTLs identified by Wissuwa (2006) or Takehisa *et al.* (2006) might be related to various existing stress factors in paddy fields, but not to specific stress factors in hydroponic culture. To elucidate the stress factors related to leaf bronzing in paddy field rice, it is essential to clarify the interrelations of between these QTLs detected in rice of paddy fields and those detected in rice grown in hydroponic or pot culture with various nutritional stresses.

Second, it is necessary to confirm the function of QTLs using CSSL or NIL in two conditions, paddy fields and laboratories, because it is not possible to clarify the precise locations and gene actions of individual QTL using only the statistical analysis (Yano and Sasaki 1997). Moreover, CSSL and NIL have a beneficial for identification of physiological factors of these QTLs. Ishimaru *et al.* (2005) suggested that a QTL for rice yield improved carbohydrate storage capacity and kept sink activity higher in the reproductive stage, and consequently increases yield potential, by analyzing physiological characters in NIL. Kashiwagi and Ishimaru (2004) indicated also that a QTL for pushing resistance of lower part deeply-involved in lodging resistance by typhoon in rice. Actually, if the difference of physiological characters in NIL and the recurrent line in the paddy field can be observed similarly in hydroponic culture with specific stresses, it can be identified that a specific stresses are a main stresses in the paddy field. Moreover, in the specific stresses conditions, comparison of the NIL and the recurrent line in the physiological characters, such as accumulation of various ions and the activity of various enzymes will lead to clarification of the physiological mechanism of leaf bronzing in paddy fields.

Third, it is necessary to promote cloning to isolate the target gene on QTL region by segregating a population for leaf bronzing and using the rice genome database. Cloning of the target gene can identify the genetic factor and genetic mechanisms of leaf-bronzing tolerance in rice. Moreover, the QTL might be effective for marker-assisted selection (MAS) to select tolerant cultivars in paddy fields.

Each second and third strategy had been effectively used to identify genetic or physiological factors of nutritional stresses in rice grown in paddy field (Wissuwa *et al.* 1998; 2006). However, as far as I know, there is not study that identified stress factors in soil, which effect rice growth in paddy field, by using QTL analysis. The tolerance mechanism of leaf bronzing including these factors could be identified only by combination of the three strategies together, with dissolution of the inconsistency. These strategies might be powerful tools to clarify the stress-related, physiological and genetic factors in paddy fields. The obtained information is expected to be useful for rice breeding of nutritional-stress-tolerant cultivars for use in paddy fields.

Finally, Wissuwa *et al*. (2006) indicated that leaf bronzing did not necessarily concur with other more important indicators of tolerance to Zn deficiency, such as Zn content per plant and mortality. The results indicated that leaf bronzing was a symptom of nutritional disorders, and it is not always reflected all disorders in paddy fields. Nevertheless, it is sure that the genetic and physiological factors of leaf bronzing related to the tolerance mechanism for nutritional stresses, because leaf bronzing is one of the nutritional disorders, and promoted by nutritional stresses. Therefore, elucidation of genetic and physiological factors for leaf bronzing in rice grown in paddy fields represents the first important step toward gaining more complete understanding of required tolerance mechanism and tolerance rice cultivars.

CONCLUDING REMARKS

The occurrence of nutrient disorders, including leaf bronzing of rice grown in paddy fields, is affected by various stress factors in soil, and physiological and genetic factors in plants. Apparently, it is difficult to clarify these factors because rice growth is affected by environmental changes such as temperature, rainfall, and soil conditions. However, QTL analysis using DNA markers in rice has made it possible to map and characterize the genetic factors underlying leaf bronzing occurred in paddy soil.

Future studies will confirm the interactions of two QTLs by using NILs, in addition to stress and physiological factors using the introduced strategies described in this review. Studies of genetic diversity of leaf bronzing in rice lead to the clarification of not only the genetic factors for leaf bronzing but also the stress factors in rice paddy fields. Furthermore, the information will contribute to the understanding of the tolerance mechanism and breeding of tolerant cultivars for cultivation in paddy fields with various nutritional stresses.

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JAPANESE ABSTRACT

イネの栄養障害の症状のひとつとして、葉身に褐色の斑点（Leaf bronzing）が発症することが知られている。これまで、Leaf bronzing の発症に関わるストレス要因、生理要因および遺伝要因について、水耕栽培やポット栽培を用い解明が試みられてきた。その結果、Leaf bronzing の発症には過剰鉄や過剰マンガン、亜鉛欠乏などのストレス要因が関与していることが明らかになり、その発症を制御する生理要因および遺伝要因が明らかにされてきた。しかし、水耕やポット栽培における過剰鉄や過剰マンガン下でも Leaf bronzing を発症しない系統が水田では発症するなど、室内で明らかにされてきた発症を抑制する機構が必ずしも野外の水田では機能していないことを示す結果も報告されている。この総説では、1950 年代から現在にいたるまで明らかにされてきたイネの Leaf bronzing の発症に関与するストレス要因、生理要因および遺伝要因を紹介し、水田におけるイネの発症に関わる要因および機構を明らかにするための研究戦略を考察する。