

Studies on the breeding of sorghum varieties resist to sheath blight (*Rhizoctonia solani* Kühn)

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Chapter 1. Introduction

The farm-management scale in Japan has increased rapidly after World War II, as affected by the standardization of the agricultural products in marketing and the concentration of the distribution system of markets, especially in the 1955's. Concurrently, the particular sections in the management, e.g., livestock farming, and farming of cereals, fruits and vegetables, have been expanded. Consequently, the farmer's income has increased more than that in the farm management system of traditional agriculture that included many small sections in a farm.

However, there are many serious problems in the modern management system. The tactics of the management system for the farmer to concentrate on the management of a particular section and abolish other sections have often resulted in overproduction of the particular products and the decrease of soil fertility in the crop fields in the case of farms without livestock^{21,57)}.

Therefore, the planning for producing the agriculture products in the region and adjustment of selling to the markets has become important. Moreover, soil management is also more important in crop production to increase and maintain a sustainable crop production.

While manure from livestock is a valuable resource for farmers growing crops, it is annoying to use or stock in the livestock farms. The fields of livestock farms sometimes become too rich in soil nutrition and causes pollution of water. The livestock farmers will need to pay the cost for cleaning the environment in the future⁵²⁾.

In livestock management in Japan, there are other serious problems for sustaining the income. The amount of roughage production in the farms is usually less than that needed for feeding the cows. The percentage of domestic roughage has decreased year by year⁴⁾, and the management of the livestock farms in Japan has become extremely unstable.

In order to stabilize and increase the income of livestock farms and to reduce the negative effects on the environment, the production of forage crops, which can absorb the large amount of nutrients released from the soil and have higher productivity for dry matter production per unit ground area and higher feeding value, will be recommended in Japan.

Sorghum is a forage crop that has excellent ability of utilizing the soil nutrients and water and realizes higher dry matter productivity even under poor soil conditions. Furthermore, it has resistance to heavy dressing of fertilizer and resistance to submerged condition; it can usually be grown without insecticides or pesticides. For this reason, I believe that cropping of sorghum will be available for solving the economical and environmental problems in livestock farms.

For the same reason, commercial varieties of sorghum have been sometime grown in the paddy fields as well as upland fields in Japan. These cases have increased because the adjustment of rice production area was needed to decrease the stock of rice grain⁴⁾. When sorghum commercial varieties are grown on a wet and fertile paddy field enriched with manure under high temperature and humid air moisture condition, sorghum plants are more susceptible to sheath blight (*Rhizoctonia solani* Kühn) and caused the lodging of the plants, consequently the dry matter yield often decreases.

However, the varieties completely resistant to the disease have not been developed yet, because the



Fig.1.1 The varietal difference of plant type among sorghum.

mechanism of resistance to the pathogen is not clear and the materials of sorghum breeding have a more wide variation in plant type (Fig.1.1) and feeding value as affected by mutant genes for increasing fiber digestibility, e.g., brown midrib (*bmr*)^{2,3,5,10,44,53} and bloomless (*bm*)^{37,53}. Especially, the field resistance seems to be more important than the true resistance because the disease can infect many crops⁵⁶ and differentiates in many anastomosis groups^{1,49} in the world. A concrete testing system needs to be developed by elucidation of the genetic mechanism and the relationships between the susceptibility and morphological characteristics and feeding value.

In this study, in order to develop the new methods of evaluation in a field inoculation testing, the field experiments were carried out to clarify the varietal differences in resistance to the sheath blight by anastomosis group 1 (AG-1), which can infect all types of sorghum (Chapter 2), and relationships between the yield loss and the degree of disease severity (Chapter 3). In Chapter 4, diallel analysis was carried out to estimate the genetic mechanism related to resistance of the disease (Chapter 4). According to the results, the effects of evaluation for resistance and field selections were verified through the field experiments that were conducted over an 18-year period (Chapter 5, 6).

These studies have consequently contributed toward development of a field inoculation system for evaluation of sheath blight and new hybrid varieties and parental inbred lines with field resistance. It was also realized that 2 new varieties, “Hazuki”^{29,30,50} and “Akidachi”⁵¹ (Fig.1.2), held sufficient field resistance



Fig.1.2 New sorghum varieties “Hazuki” (left) and “Akidachi” (right).

to sheath blight in spite of having a higher digestibility of the fiber.

Chapter 2. Varietal difference of resistance to sheath blight

For the improvement of the resistance to *Rhizoctonia* sheath blight, sorghum breeders have been screening the resistant crops by field inoculation test, using anastomosis group 1 (AG-1) isolated from sorghum during the past 15 years²²⁻²⁷.

In the field inoculation test with AG-1, the resistance to the disease varied with the variety of sorghum²² and maize^{13,31}). However, sorghum varied more widely than maize in the plant height and yield of the grains, depending on the use. The varietal difference of resistance to the disease in the field and the criteria for the evaluation of the resistance common to all plant types were not yet clarified. There have been no reports on the difference of resistance to the disease among the varieties of sorghum including all plant types.

The objectives of this chapter are 1) to find the varietal difference in the resistance to *Rhizoctonia* sheath blight by a field inoculation test, and to find the resources of the resistance, using the varieties of all types of sorghum ; and 2) to establish the common criterion for evaluation of the resistance.

2.1. Materials and methods

A field inoculation test was carried out at the field of Nagano Animal Industrial Experiment Station in 1984 and 1985. In 1985, 72 varieties including all plant types classified by the objective of use were examined (Table 2.1). The varieties of Indian type were selected from the group of sorgho type for improving the yield of grain by ICRISAT. The varieties of Korean type are distributed in North China and Korea and are slender in shape.

Thirty-one typical varieties were also selected from each plant type and their resistance to the disease was evaluated in 1984 and 1985. Barnyard manure and chemical fertilizer of N, P₂O₅ and K₂O were applied at the rate of 30,000 kg, 120 kg, 120 kg and 90 kg per ha, respectively, in both years. Half of the N chemical fertilizer was applied as ammonium sulfate at the middle of the vegetative growth period.

The varieties were sown with an interhill spacing of 8 cm and interrow spacing of 75 cm from the middle to the end of May. Twenty-five plants per plot were used for each line. AG-1^{40,54} which was the most pathogenic to sorghum and maize was obtained from sorghum by the National Grassland Research Institute, and incubated for two weeks after inoculation onto barley grains. After 2 months of seeding, the grains were spread on the hill and covered with a thin layer of soil³³). Resistance to the disease was evaluated before harvesting from the middle of September to early in October. The height of lesion (HL)

Table 2.1 Materials of the field inoculation test for *Rizoctonia* sheath blight.

Plant type	Number of cultivars	Height of flag leaf collar at harvest time (cm)	
		Mean	s.d.
Grain type	26	77.4	22.1
Dual-purpose type (F ₁)	12	133.2	36.3
Sorgho type	6	205.3	34.7
Indian type	11	141.0	19.2
Korean type	4	144.8	53.7
Male sterile line	13	81.8	23.0
Total	72	111.4	48.2

and the height of flag leaf collar (HF) were measured at maturity. To simplify the process of further screening, the relative lesion height (RLH)²²⁾ was used as an indicator of the resistance to the disease.

$$RLH = \frac{(\text{height of lesion on the sheath})}{(\text{height of the flag-leaf collar})} \times 100 (\%)$$

2.2. Results

If the order of the resistance to the disease of sorghum varieties varies markedly with the year, the field test must be repeated each year to identify accurately the varietal difference. Therefore, in this study, the field experiments with 31 varieties, which contained each plant types, were conducted in 1984 and 1985. Fig.2.1 shows the change of daily mean temperature in each year. The difference between two years was within 2°C from inoculation time to investigation. Analysis of variance was carried out for HL, RLH and HF (Table 2.2). The difference among varieties was significant in all characters ($p < 0.001$), but no difference between years was in HL and RLH. In HF, there were also detected the differences between

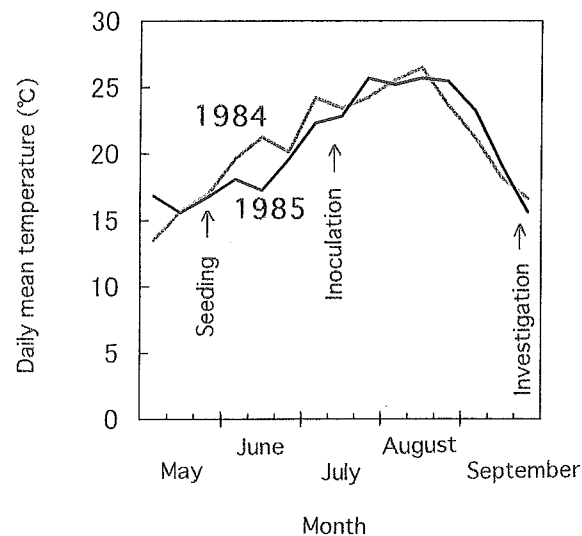


Fig.2.1 Change of daily mean temperature.

Table 2.2 Effects of variety and year on the results of the field inoculation test (ANOVA).

Factor	d.f.	M.S.		
		Height of the flag leaf collar (HF)	Height of lesion on the sheath (HL)	Relative lesion height (RLH)
Block	1	386**	24	11
Variety (V)	30	5567***	619***	2296***
Year (Y)	1	765***	452.0	141.0
V × Y	30	136***	175	113*
Error	61	37	210	67

*** : $p < 0.001$, ** : < 0.01 , * : $p < 0.05$

Table 2.3 Correlation coefficient in each trait between 1984 and 1985.

Trait	Correlation coefficient (n=31)	Significance
Height of the flag leaf collar (HF)	0.957	$p < 0.001$
Height of lesion on the sheath (HL)	0.732	$p < 0.001$
Relative lesion height (RLH)	0.909	$p < 0.001$

years and interaction between variety and year ($p < 0.001$). The correlation coefficients between the values obtained in 1984 and 1985 are shown in Table 2.3. There was a significant correlation in each value (HL, HF and RLH) between the two years ($p < 0.001$).

There was no significant correlation between maturity of the varieties and HL or RLH in whole data, and also no correlation in each type.

RLH was closely correlated with the degree of yield loss, and it was thought to be an index of the degree of damage by the disease²²⁾. The relationship between HL, which shows the disease severity, and RLH, which shows the yield loss and quality damage, is shown in Fig.2.2. The higher the HL, the higher was the RLH of the variety. Among the varieties with the same HL, the RLH was higher in the grain-type sorghum than in the other types. The three groups were distinguished from each other by statistical comparison of the regression equations between HL and RLH. The regression line A in Fig.2.2 was obtained from the grain-type and male-sterile lines, line B from the dual-purpose and Korean types and line C from the sorgo-type including the Indian-type. The slope of the regression line was the largest in A followed by B and C, in this order. Each regression equation was highly significant ($p < 0.001$).

Because plant height was correlated with the degree of damage that could be expressed by RLH, the relationship between HF and RLH was analyzed (Fig.2.3). The regression equations between HF and RLH were also classified into the same three groups as that between HL and RLH. In group A, the slope was the steepest and the RLH greatly varied even in the varieties with the same HF. Contrary to group A, the RLH in group C was similar irrespective of HF. The variation in the degree of damage by the disease (RLH) was larger in group A than in the other groups.

No correlation was observed between HF and HL (Fig.2.4).

2.3. Discussion

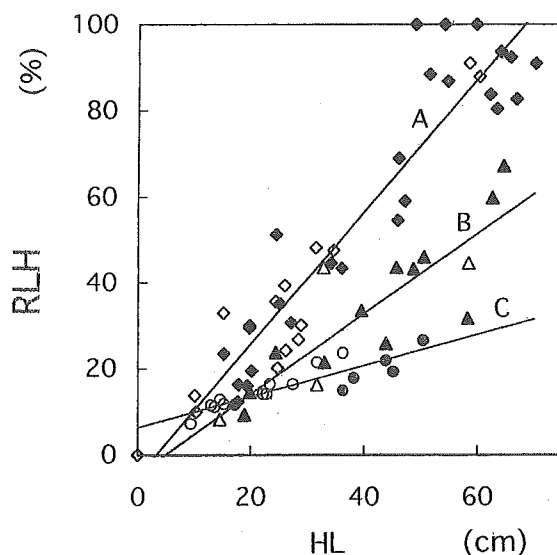


Fig.2.2 Relationship between RLH and height of lesion on the sheath (HL).

◆ : grain type, ▲ : dual-purpose type (F_1), ○ : Indian type,

△ : Korean type, ◇ : male sterile line, ● : sorgo type.

A: Regression from grain and mail sterility types.

$$y = 5.22 + 1.54 \cdot x (r = 0.94, p < 0.001).$$

B: Regression from dual-purpose (F_1) and Korean types.

$$y = 4.61 + 0.94 \cdot x (r = 0.87, p < 0.001).$$

C: Regression from Indian and sorgo types.

$$y = 6.31 + 0.36 \cdot x (r = 0.89, p < 0.001).$$

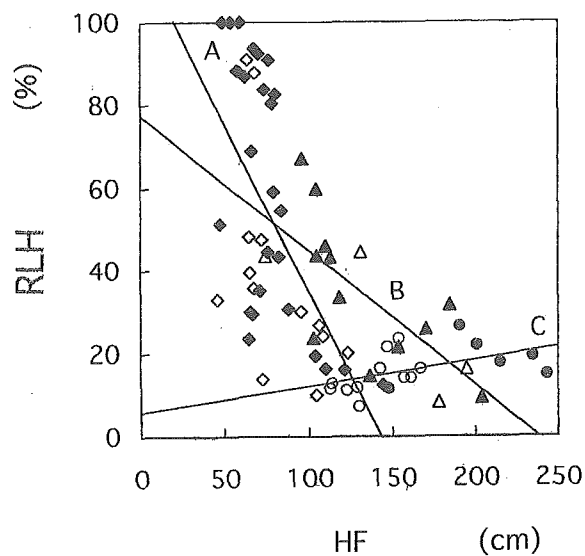


Fig.2.3 Relationship between RLH and height of the flag leaf collar (HF).

◆ : grain type, ▲ : dual-purpose type (F_1), ○ : Indian type,
 △ : Korean type, ◇ : male sterile line, ● : sorgo type.

A: Regression from grain and mail sterility types.

$$y = 116.40 - 0.82 \cdot x (r = -0.59, p < 0.001).$$

B: Regression from dual-purpose (F_1) and Korean types.

$$y = 77.56 - 0.33 \cdot x (r = -0.74, p < 0.01).$$

C: Regression from Indian and sorgo types.

$$y = 5.82 + 0.07 \cdot x (r = 0.50, p < 0.05).$$

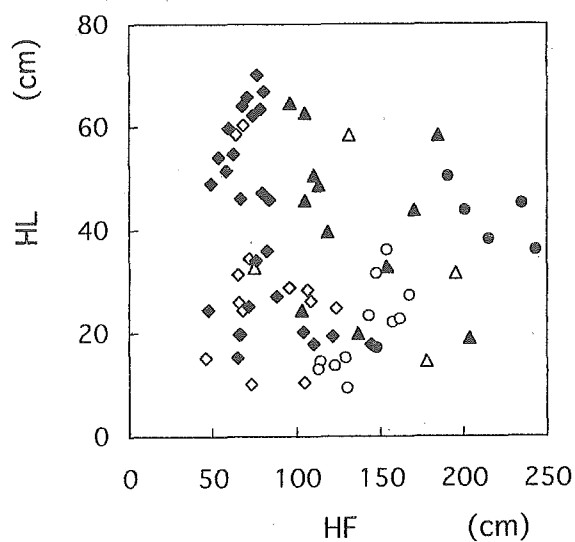


Fig.2.4 Relationship between height of lesion on the sheath (HL) and height of the flag leaf collar (HF).

◆ : grain type, ▲ : dual-purpose type (F_1), ○ : Indian type,
 △ : Korean type, ◇ : male sterile line, ● : sorgo type.

HL, HF and RLH obtained in 1984 were significantly correlated with those obtained in 1985 ($p < 0.001$). Air temperature has been reported to affect the degree of infection with *Rhizoctonia solani* Kühn⁴⁶. If the effect of temperature on the degree of the infection varies with the variety, the order of the resistance of each variety may vary with the year, and the correlation coefficient between years may be low. However, the variance among varieties was higher significant in HL, RLH and HF from the results of ANOVA, while the variance between years and that of interaction between variety and year were not or less significant in HL and RLH. Furthermore, a significant correlation was observed between the two years in three characters. From these results, the order of the resistance of each variety is not considered to vary with the meteorological factors of the year. Thus, it is supposed that the varietal difference of the resistance to the disease can be compared with the data obtained in a single year. Hence, in this study, the varietal difference is discussed based on the results of 1985 alone.

The significant correlation was not observed between HF and HL. This suggests that the severity of the disease is not correlated with the plant size.

The high ratio of total carbon to total nitrogen content in stover tended to increase the resistance to the *Rhizoctonia* sheath blight in rice¹⁹. Plant nutritional condition has been considered to affect the resistance. In sorghum, the grain yield widely differs with the variety. Therefore, the varieties with high grain yield may contain a smaller amount of carbon in stover than in the varieties with a low grain yield, and may be susceptible to the disease. In this experiment, the male sterile lines with a low grain yield tended to have a higher resistance to the disease than the other varieties with a high grain yield. On the contrary, the Indian-type varieties with a high grain yield tended to have a higher resistance than the sorgo-type varieties that yielded no grain. This conflicting phenomenon suggests that some other factors are also related to the resistance in addition to plant nutritional condition.

RLH is an important marker for the severity of injury caused by the infection with *Rhizoctonia* sheath blight, because there was a high positive correlation between RLH and yield loss ($r = 0.816$, $p < 0.001$)²². Rice varieties have been evaluated and screened by the severity of injury at the first stage of breeding¹⁸, and relative lesion height has been used to simplify the method of evaluation from the late 1980s^{19,20}. The relative lesion height is similar to RLH in our research. In sorghum, the average HF of each plant type widely varied from 77 cm to 205 cm (Table 2.1), and the relative indicator such as RLH should be used to evaluate the resistance.

In rice, it was pointed out that the disease severity evaluated by RLH was not different among varieties, but that the severity evaluated by the ratio of the number of infected tillers to the total number of tillers was significantly different²⁰. In the sorghum used in this study, however, the tiller number was less than two per plant. Another method for the evaluation of the disease severity will be necessary for screening of the varieties with many tillers such as Sudan type sorghum and Sudangrass.

The RLH of the varieties in group A (grain type) widely varied among varieties that had similar HF, suggesting that the genetic diversity of the resistance to the sheath blight exists within the group.

When screening rice for sheath blight resistance, the plants with an RLH of less than 20% were considered to be resistant, and 7,614 varieties and lines were evaluated in 1987. Many resistant lines were found in wild rice and upland rice²⁰. In this field test for sorghum, the resistant varieties with an RLH of less than 20% were also found. However, the resistant varieties did not belong to the specific types. It seems to be favorable to breed the resistant varieties for each type independently.

The resistant varieties should have low disease severity, low yield loss and little quality damage. The resistance to infection (disease severity) may be evaluated by HL, and the degree of yield loss and quality damage by HL and RLH. Therefore, the resistance of varieties in the field may be evaluated effectively based on the relationship between HL and RLH^{22,23,27}.

Fig.2.2 shows the relationship between HL and RLH. The degree of yield loss and quality damage (RLH)

relative to the disease severity (HL) were severer in the varieties with high grain yield than in those with low grain yield such as sorgho type varieties. It is important in sorghum that the slope of the regression line between RLH and HL varied with the plant type. It seemed that there was no common criterion for the selection of the resistance to the sorghum sheath blight from all plant types. However, the intercepts of the regression lines were similar in all types, and most of the varieties with an HL of less than 20 cm showed an RLH of less than 20%, irrespective of the plant type. KASUGA observed that the yield loss was slight in the varieties with an HL of less than 20 cm (unpublished). Furthermore, the lower the RLH, the lower the yield loss²²⁻²⁴). Therefore, a common criterion for the selection of the resistance to the sorghum sheath blight in a field inoculation test may be an RLH of less than 20 % and HL of less than 20 cm for all plant types.

2.4. Summary

To search for genetic resources of resistance to sorghum sheath blight, I examined the varietal difference of the resistance by a field inoculation test. The materials used were 72 varieties and lines, which were classified into grain, dual purpose (F₁) and sorgho types, including the Indian-type varieties selected for grain yield from grain type, Korean varieties and male-sterile lines. Field inoculation tests were carried out in 1984 and 1985. Barley grains were inoculated with anastomosis group 1 (AG-1), which was the most pathogenic to sorghum, and the grains were spread on the hill and covered with soil two months after the seeding of the test plants. Resistance to the disease was evaluated by the relative lesion height (RLH), which is the ratio of the height of lesion on the sheath (HL) to the height of flag leaf collar at maturity (HF). The varietal differences of HL, HF and RLH were similar in both years, and the coefficients between the values obtained in the two years showed a significant correlation ($p < 0.001$). HL showing the degree of invasion and RLH showing the damage of plants closely correlated with each other, and the relationship in each of the three groups, grain type including male sterile lines, dual-purpose type including Korean type and sorgho type including Indian type, was regressed to a linear equation. The correlation between HF and RLH in each of the same three groups was also regressed to a linear equation ($p < 0.001$). A wide variation in RLH was detected among the varieties of grain type with the same plant height. The higher resistant resources were found in every type, and a common criterion of the resistance was considered to be an RLH of less than 20% and HL of less than 20 cm.

Key word : inoculation, resistance, *Rhizoctonia solani* Kühn, sheath blight, sorghum, variety

Chapter 3. Relationships between the yield loss and the resistance to sheath blight

The cropping area of forage sorghum has recently increased in humid and fertile fields in Asia, but the yield and forage quality of sorghum are often damaged seriously by sheath blight (*Rhizoctonia* sheath blight)^{24,42}.

Especially, the lodging and damping-off of forage sorghum by sheath blight is frequently observed in Japan. The pathogen, *Rhizoctonia solani* Kühn, infects not only sorghum but also rice²⁰, maize⁴⁰ and soybean⁴¹.

The most economical and effective method of controlling the disease without agricultural chemicals is the development of varieties resistant to sheath blight. However, there have been no reports about the yield loss of sorghum by sheath blight in Japan.

The objectives of this chapter are 1) to clear up the relationship between the degree of the yield loss in sorghum by sheath blight and the degree of the resistance to the disease and 2) to establish the common criterion for evaluation of the resistance in sorghum.

3.1. Materials and methods

A field inoculation test was carried out at the field of Nagano Animal Industrial Experiment Station in 1985. Seventy-two varieties including grain types, dual purpose types and sorgho types by objective of use were examined (Table 3.1). Barnyard manure and chemical fertilizer of N, P₂O₅ and K₂O were applied at the rate of 30000, 120, 120 kg and 90 kg per ha, respectively. Half of the N fertilizer was applied as ammonium sulfate at the middle of the vegetative growth period. The varieties were sown with an interhill spacing of 8 cm and interrow spacing of 75 cm on May 17. Fifteen plants per line were inoculated. The most pathogenic race to sorghum and maize, AG-1⁵⁴, that had been obtained from sorghum at the National Grassland Research Institute, was incubated for two weeks after inoculation onto barley grains. After 2 months of seeding²⁵, the grains were spread on the hill and covered with a thin layer of soil⁵⁴.

Resistance to the disease was evaluated at harvesting time in early October. The height of lesion on the sheath (HL) and the height of flag-leaf collar (HF) were measured at maturity. The relative lesion height (RLH)^{22,23} was calculated as an indicator of the resistance to the disease and the degree of damage.

$$RLH = \frac{(\text{height of lesion on the sheath, HL})}{(\text{height of the flag-leaf collar, HF})} \times 100 (\%)$$

Furthermore, dry matter weight of 5 plants which were inoculated and non-inoculated respectively per each line were measured at maturity. The relative dry matter weight (RDM)^{22,24} was calculated as an indicator of the yield loss by the disease and the degree of damage.

$$RDM = \frac{(\text{dry matter weight of inoculated 5 plants})}{(\text{dry matter weight of non-inoculated 5 plants})} \times 100 (\%)$$

3.2. Results

The environment after inoculation was favorable enough for disease development. Plant length, culm length, height of the flag leaf collar, panicle length and neck length of panicle of inoculated plants were smaller than non-inoculated plants significantly at the 1 % level (Table 3.1).

The height of lesion (HL) of 72 varieties varied from 9 cm to 70 cm, and the relative lesion height (RLH) varied from 7.4% to 100%. Furthermore, the height of flag-leaf collar (HF) varied from 46 cm to 244 cm (Table 3.2).

Table 3.1 Effects of the inoculation for *Rhizoctonia solani* Kühn on the morphological characteristics.

Treatment	Plant length cm	culm length cm	Height of the flag leaf collar cm	Panicle length cm	Neck length of panicle cm
Inoculated	157 ^A	123 ^A	112 ^A	25 ^A	11 ^A
Non-inoculated	164 ^B	131 ^B	119 ^B	26 ^B	12 ^B

The values are the mean of 72 varieties.

Means with different letter in each column are significantly different. A and B at p<0.01.

Table 3.2 Varietal difference of resistance to sheath blight in inoculation test.

Item	Height of lesion on the sheath (HL) cm	Relative lesion height (RLH) %	Height of the flag leaf collar (HF) cm
Mean	36	39.6	111
Maximum value	70	100.0	244
Minimum value	9	7.4	46
Standard deviation	17.3	28.3	48.2

Table 3.3 Relative dry matter weight (RDM) of inoculated plants.

Item	RDM		
	Panicle (A) %	Stover (B) %	Whole plant (A+B) %
Mean	72.0	76.7	74.6
Maximum value	100.0	100.0	100.0
Minimum value	13.5	25.0	24.8
Standard deviation	25.1	19.8	22.2

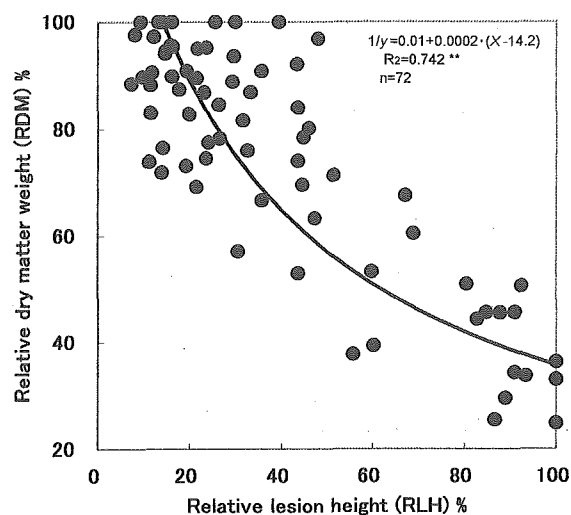


Fig.3.1 Relationship between relative lesion height (RLH) and relative dry matter weight (RDM) in Rhizoctonia sheath blight.

The relative dry matter weight (RDM) varied from 25% to 100%. And average RDM of 72 varieties was 74.6%. When it is seen from the viewpoint of part in plant, the average RDM of head was 4.7 points smaller than the average RDM of stover (Table 3.3).

Fig. 3.1 shows the relationship between RLH and RDM of 72 varieties. The RDM tended to be depressed by increasing RLH. The relationship between RLH and RDM fitted the reciprocal model significantly ($p < 0.001$). The reciprocal model for RDM, as based on the relationship between RLH (x) and RDM (y), was as follows: $1/y = 0.01 + 0.0002(x - 14.2)$.

Therefore, the yield loss was recognized at RLH of 14.2% in the reciprocal model.

3.3. Discussion

PASCUAL *et al.* (1988)⁽⁴²⁾ reported the grain yield loss in sorghum due to Rhizoctonia sheath blight in the Philippines. In the report, Rhizoctonia sheath blight was estimated to reduce grain yield from 35% to 43%.

In this experiment, the range of the relative dry matter weight (RDM) of panicle was broad from 13.5% to 100%. RDM of stover and weight of whole plant were broad from about 25% to 100% (Table 3.3). Therefore, the yield loss of panicle in sorghum was from 0% to 86.5%. The yield loss of stover and weight of whole plant were broad from 0% to about 75%.

The range of severity levels in this experiment was enough to find the relationships between the yield loss and the resistance to sheath blight in sorghum.

It is important to explain the relationships between the yield loss and the resistance to sheath blight for developing the varieties, which resist to the disease. Therefore, the RLH at which the dry matter yield starts decreasing is an important criterion for selection in the field inoculation test.

On the other hand, from the relationships between RLH and HL, I pointed out that the criteria for resistant varieties are HL of less than 20 cm and RLH of less than 20 % in the field inoculation test for all sorghum types²⁸⁾ (See Chapter 2).

In this experiment, from analysis of reciprocal model, the RLH at which the dry matter yield starts decreasing is estimated about 14.2%.

From these results, the selection by the criteria of the resistance, which is considered to be RLH of less than 20% and HL of less than 20 cm from the relationship between RLH and HL, is effective from the viewpoint of the relationship between RLH and RDM.

3.4. Summary

To clarify the selection criteria of the resistance to sheath blight (*Rhizoctonia solani* Kühn) in sorghum, a field inoculation test was carried out in 1985. The materials used were 72 varieties and lines, which were classified into grain types, dual-purpose types and sorgo types. Barley grains were inoculated with anastomosis group 1(AG-1), which was the most pathogenic to sorghum, and the grains were spread on the hill and covered with soil two months after seeding. Resistance to the disease was evaluated by the relative lesion height (RLH), which is the ratio of the height of lesion on the sheath (HL) to flag leaf collar (HF) at maturity. Yield loss by the disease was evaluated by the relative dry matter weight (RDM), which is the ratio of the dry matter weight of inoculated plants to non-inoculated plants.

The HL of 72 varieties in inoculated plants varied from 9 cm to 70 cm, and the RLH varied from 7.4% to 100%. The RDM varied from 22% to 100%. There were varietal differences in the yield loss by sheath blight among 72 varieties.

The relationship between RLH and RDM was fitted reciprocal model significantly ($p < 0.001$). The reciprocal model for RDM, as based on the relationship between RLH (x) and RDM (y), was as follows: $1/y = 0.01 + 0.0002(x - 14.2)$.

The common criteria of the resistance, which are considered to be RLH of less than 20% and HL of less than 20 cm from the relationship between RLH and HL, is effective from the viewpoint of the relationship between RLH and RDM.

Key word: reciprocal model, yield loss

Chapter 4. Diallel analysis of resistance to sheath blight

The cropping area of forage sorghum has recently increased in humid and fertile field in Asia, but the yield and forage quality of sorghum were often damaged seriously by sheath blight (*Rhizoctonia* sheath blight)^{22,24)}.

One of the favorable ways to suppress the disease without agricultural chemicals is the development of varieties resistant to sheath blight. For the development of the resistant varieties, KASUGA *et al.*^{22,23,25)} screened the various inbred lines and F₁ varieties by field inoculation test during the past 15 years and reported the varietal differences for the resistance to sheath blight on the basis of various indices and the results of the selection of resistant inbred lines.

Practically, the genetic analysis is necessary to breed the highly resistant strains in the field to a wide range of races of the disease, because varieties with only one major resistant gene may be infected with a newly developed race of the disease with high probability. In the case of resistance to southern corn leaf blight (*Bipolaris maydis* (Nishikado and Miyake) Shoemaker), it was revealed by diallel analysis that multiple genes controlled the resistance³²⁾.

In sorghum sheath blight, SHAUG *et al.* reported that the heritability of the resistance which was expressed by the relative lesion height ($[\text{height of infected sheath}]/[\text{height of flag leaf}]$: RLH) was highly

significant and the gene actions showed additive dominant effects⁴⁶⁻⁴⁸). However, the report did not describe plant type, which the materials belong to. Morphological variation among sorghum varieties is extremely large²²⁻²⁶) and the varieties could be classified into three groups of plant types by the height of lesion on the sheath (HL) and the degree of damage (RLH)²⁷), recently. Therefore, the analysis should be carried out for each group and by considering the two characteristics.

The objectives of this chapter are 1) to analyze the inheritance of the resistance to sheath blight in view of height of lesion on the sheath (HL), the degree of damage (RLH) and plant size (the height of flag leaf : HF) by all possible reciprocal crosses of 5 lines in grain type group, in which varietal difference for the resistance is larger than other groups²⁷), and 2) to clarify the effect of the combining ability for establishing the breeding system to obtain the lines with a high field resistance to the disease.

4.1. Materials and methods

Five inbred lines were used (Table 4.1), which belong to grain type but differ in the resistance to sheath blight, and 20 hybrids and 5 inbred lines were obtained by the diallel cross of these lines. A field inoculation test was carried out in Nagano Animal Industrial Experiment Station in 1991. The experiment was designed as randomized block with two replications. Barnyard manure and chemical fertilizer of N, P₂O₅ and K₂O were applied at the rate of 30000, 120, 120 kg and 90 kg per ha, respectively. Half of the N fertilizer was applied as ammonium sulfate at the middle of the vegetative growth period. The inbred lines and hybrids were seeded on May 29, and were established one plant per hill with an 8-cm interhill spacing and 75-cm interrow spacing. Twenty-five plants per plot were used for each line. The most pathogenic race to sorghum and maize, AG-1⁵⁴), that had been obtained from sorghum at the National Grassland Research Institute, was incubated for two weeks after inoculation onto barley grains. After 2 months of seeding, the grains were spread on the hill and covered with a thin layer of soil³³).

Resistance to the disease was evaluated at harvesting time, from mid-September to early October. The height of lesion on the sheath (HL) and the height of flag-leaf collar (HF) were measured at maturity. The relative lesion height (RLH)²²) was calculated as an indicator of the resistance to the disease and the degree of damage.

$$RLH = \frac{(\text{height of lesion on the sheath, HL})}{(\text{height of the flag-leaf collar, HF})} \times 100 (\%)$$

The diallel data were *arc sin* transformed and then analyzed according to the model proposed by HAYMAN^{11,12,39,45}). The parameters of diallel analysis were calculated by the computer program written by UKAI⁵⁵).

4.2. Results

Table 4.1 shows characteristics of the parental lines used in the diallel cross. Total culm length of all parental lines was smaller than 150 cm. The range of heading date between the earliest line S.D.102 and

Table 4.1 Characteristics of the parental lines used in the full diallel analysis.

No.	Line	Origin	Heading date	Total culm length (cm)	Height of flag leaf collar : HF (cm)	Height of lesion on the sheath : HL(cm)	Relative lesion height : RLH (%)
1	S.D.102	U.S.A.	14-Aug	80	50.0	50.0	100.0
2	BJ12	Mexico	17-Aug	105	86.0	40.0	48.4
3	I.S.517	India	16-Aug	111	94.0	58.0	62.8
4	M36001	India	18-Aug	122	121.0	22.0	18.4
5	M91034	India	24-Aug	143	139.0	14.0	10.1

Table 4.2 Analysis of variance for RLH, HL and HF in 5×5 full diallel tables.

Item	d.f.	M.S.		
		RLH	HL	HF
a	4	2249.4**	1412.8**	13166.5**
b	10	218.5	108.7	3086.4
b ₁	1	529.0**	2.1	11077.6**
b ₂	4	87	53.9	2794.0**
b ₃	5	261.5**	173.9	1722.2**
c	4	44.2	52.3	25.5
d	6	38.7	59.5	7
Error	24	37.1	81	13.3

** : Significant at 1% level.

RLH, HL and HF : See Table 4.1

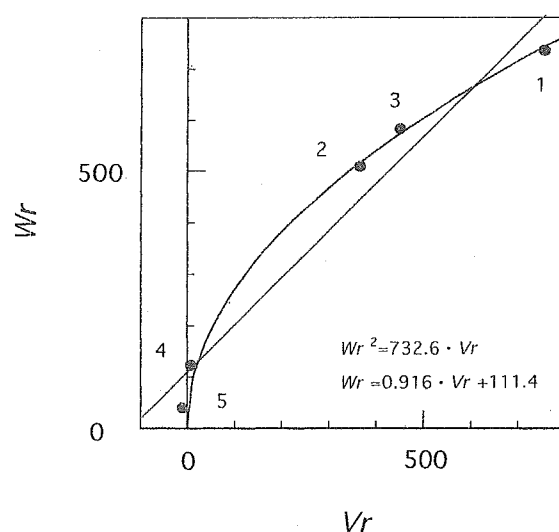


Fig.4.1 W_r/V_r graph for RLH in 5×5 diallel cross.

W_r : the covariance of between the parents and their offsprings in the r th array ; V_r : the variance of the r th array.

No. in figure : See Table 4.1.

the latest line M91034 was 10 days. The tiller was not observed in any lines.

Table 4.2 shows the results of the analysis of variance for RLH, HL and HF. For RLH, the effects of “a” which mean additive effects, “b₁” which mean dominance effects and “b₃” which mean residual dominance effects appeared in a specific combination, respectively were significant at the 1 % level. The reciprocal effects in diallel crosses (“c” and “d”) were not significant.

The variance and covariance graph for RLH shown in Fig.4.1, provides information about the genetic relationship among parental lines. A slope of the linear regression of W_r on V_r was nearly equal to 1, which indicated that there was less effect of epistasis. The intercept value of the regression line on the W_r axis shows the average level of dominance to sheath blight. From the information for the position of each parent, it was indicated that M36001 and M91034 carried more dominance genes and S.D.102 carried more recessive genes.

Fig. 4.2 shows the scatter plotting diagram between $W_r + V_r$ and RLH of parental lines (P_r) in the diallel cross. A significant positive correlation ($r=0.961$, $p<0.01$) indicated that the lines with many dominance genes expressed the lower RLH.

The genetic parameters for RLH fitted the additive and dominance model without any epistatic effect.

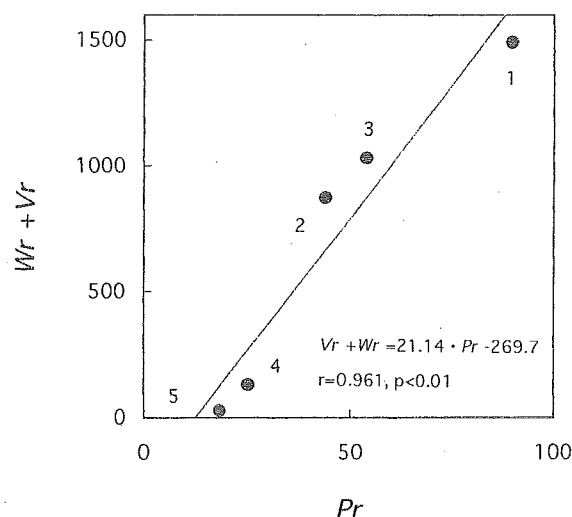


Fig.4.2 Correlation between $Wr + Vr$ and RLH of parental lines in 5×5 diallel cross.
No. in figure : See Table 4.1.

Table 4.3 Genetic parameters for RLH in 5×5 diallel tables.

Item	M.S.		
	RLH	HL	HF
D	732.6	261.5	1148.0
F	-127.4	-287.6	-2444.9
H ₁	392.0	39.1	7814.6
H ₂	369.2	61.9	6147.3
E	37.1	81.0	13.3
(H1/D) ^{1/2}	0.732	0.387	2.609
uv	0.235	0.395	0.197
Heritability			
narrow sense	0.773	0.732	0.629
broad sense	0.935	0.775	0.997

Therefore, the genetic parameters could be estimated according to the statistical genetic model (Table 4.3). An additive effect (D) was stronger than the dominance effect (H₁), and dominance effect of H₂, which was the sum of the effects of all loci in heterozygous phase in all crosses. The mean degree of dominance (H1/D)^{1/2} was estimated to be 0.732 and the average frequency of dominant and recessive genes (uv) was 0.235. Heritability was high in both narrow and broad sense.

In the analysis of variance for HL, the additive effect ("a" in Table 4.2) was significant at the 1 % level, while a dominant effect ("b" in Table 4.2) was not. In addition, there was also a slight difference between the heritabilities in narrow and broad senses shown in Table 4.3.

In the case of HF, additive effect ("a" in Table 4.2), the mean dominance effect ("b₁" in Table 4.2), the dominance effect appeared in specific parent ("b₂" in Table 4.2) and the residual dominance appeared in specific combination ("b₃" in Table 4.2) were significant at the 1 % level. However, the slope in the regression line of Wr on Vr was 0.449. This means that the results of HF did not fit the additive and dominance model. Heritability of HF was 0.629 in a narrow sense and 0.997 in a broad sense, but the difference between these values was larger than that of RLH or HL.

There was significant correlation ($r=0.942$, $p<0.001$) between the averages of better resistant and mid

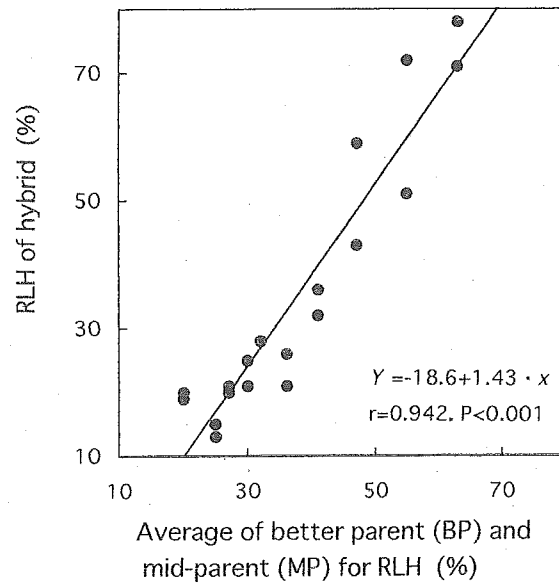


Fig.4.3 Correlation between hybrids and the parents for RLH in diallel crosses.

parent $((BP+MP)/2)$ and the hybrids for RLH (Fig.4.3).

4.3. Discussion

SHAUG *et al.* (1988)^{47,48)} investigated the genetic mechanisms of the resistance to sorghum sheath blight by half diallel analysis using maintainers (B-line) of lines with cytoplasmic male sterility as materials. The total culm length was not described in the reports, but the lines seemed to belong to the grain type. The degree of damage by the disease (RLH) was from 14 to 57% in the experiment at Taipei. The range of severity levels among parents in these full diallel experiments was broad from 10.1 to 100%. Therefore, the genetic mechanisms can be analyzed more reliably by these results in addition to their reports.

From the diallel analysis for RLH, it is suggested that the resistance to sorghum sheath blight is controlled by the multiple genes and that the genes have additive and partially dominant effects. Therefore, it is expected that varieties with many dominant genes show a lower RLH value and have higher resistance to the disease. SHAUG *et al.* (1988)⁴⁸⁾ pointed out that the resistance to this disease has quantitative characteristics and the genes have additive and dominance effects based on the half diallel analysis. PASCUAL *et al.*⁴⁹⁾ indicated that the gene effects are important in the expression of quantitative resistance to *Rhizoctonia solani* Kühn by the comparison among the RLH of parents, F_1 , F_2 and the backcrosses to each parent. The results of the full diallel analysis in this study were similar to their reports for RLH.

Furthermore, "uv" which indicates the average frequency of dominant and recessive genes was 0.235. The value suggested that the frequency of the genes were not equal and the frequency of recessive genes was higher than that of dominant genes. In SHAUG's report, the degree of average dominance and heritabilities in both the narrow and broad sense were extremely similar to our results. The higher additive effects and heritability of RLH are advantageously generated in the course of the development of the inbred lines having higher resistance to the disease.

The positive correlation between parents and hybrids were highly significant for RLH (Fig.4.3). In the resistance to southern corn leaf blight (*Bipolaris maydis* (Nishikado and Miyake) Shoemaker), KOINUMA and MOCHIZUKI³²⁾ observed that there was a significant correlation between parental lines and their F_1 hybrids in the disease severity when the average of the better resistant parent (BP) and the mid parent

(MP) was used as the parental value for the index of disease severity. The resistance to sheath blight is also similar to that to southern corn leaf blight, and the resistance in better parent affects strongly the resistance in F_1 generation for RLH. The results of diallel analysis also supported the dominance effects of the resistance. In addition, the mean square value of "a" which means general combining ability (GCA) for RLH was higher than the value of "b₃" which means residual dominance appeared in a specific combination (specific combining ability : SCA). Therefore, the degree of resistance to the disease in F_1 can be forecasted from the value of $(BP+MP)/2$ for RLH. The information may be valuable for deciding the combination in an effective breeding program.

In this experiment, the total culm length in the inbred lines, which belong to the grain type, was shorter than 150 cm. Therefore, the range of the height of flag leaf collar among the lines was narrow, and was about 89 cm. However, HL of I.S.517, which is a semi-resistant line for RLH, was higher than that of S. D.102, which is a susceptible line for RLH. This phenomenon indicates that the resistance to the disease cannot be evaluated from HL alone, even if the inbred lines belong to the grain type. In the breeding of the resistance to sorghum sheath blight, the additive effects are stronger than dominance effects for RLH, therefore the selection for RLH in early generation seems to be also effective to develop inbred line rapidly.

The highly resistant hybrid will be developed securely by the screening of parental line for RLH in addition for HL from early generation and also by combination of crossing among the resistant parental lines which express higher GCA for RLH.

4.4. Summary

Diallel analysis of the resistance to sheath blight was performed using 5×5 reciprocal crosses of lines belonging to the grain type by a field inoculation test to clarify the inheritance of the resistance to sheath blight. The mean additive effect, mean dominance and remaining dominance appearing in a specific combination were significant for relative lesion height : RLH at the 1 % level of probability. The results of the analysis for RLH could fit the additive and dominant model without epistasis effect. The heritability was high in a narrow (0.773) and broad sense (0.935). Additive effect for height of lesion on the sheath : HL was significant at the 1 % level, while the dominant effect was not. The results of the analysis for the height of flag leaf : HF did not fit the additive and dominant model. There was a significant correlation between the average RLH of better resistant and mid parent $((BP+MP)/2)$ and that of the hybrids ($r = 0.942$, $p < 0.001$). The additive effect was stronger than dominant effect for RLH. Therefore, the selection for RLH in early generation is effective to develop inbred lines rapidly.

Key word : combining ability, diallel analysis, heritability

Chapter 5. Evaluation of resistance to sheath blight by field inoculation test and improvement of the resistance by selection

In Japan, the isolates of anastomosis group 1 (AG-1) from sorghum and other crops are known to infect maize, which then wilt at the mature stage⁴⁰⁾. In a field inoculation test with AG-1, the resistance to the disease varied with the variety of sorghum²²⁾ and that of maize¹³⁾. Therefore, the resistance is genetically controlled and improvement by screening the hybrids in a field inoculation test is useful.

Field inoculation is commonly a good breeding method for the selection of disease-resistant plants because it simulates the infection of the host with the pathogen under natural conditions. However, to our knowledge, no hybrid lines, which have resistance to *Rhizoctonia* sheath blight of sorghum, have been established by field inoculation tests. The objectives of this chapter are 1) to confirm the increase of resistance of sorghum to *Rhizoctonia* sheath blight by selection with a field inoculation test, and 2) to

assess the specific combining ability for evaluating the effectiveness of selection according to the results of the field inoculation test.

5.1. Materials and methods

Materials

F₂ plants from 3 cross combinations, F6-3A-5×Senkinshiro, F6-3A-5×M36001 and M91034×Waikai(1), were obtained in the field of Nagano Animal Industrial Experiment Station (Table 5.1). In this cross, F6-3A-5 and M91034 were resistant to sheath blight pathogen. Senkinshiro and Waikai(1) are Korean type cultivars for grain and forage use and susceptible to the pathogen. Senkinshiro is the pollen parent of the commercial variety, Suzuho. In 1985, the crossing of 3 combinations were carried out and 200 individuals were propagated in the F₂ generation for each combination. The selection by field inoculation test was started in 1987.

Field inoculation test

The selected lines were sown with interhill spacing of 8 cm and interrow spacing of 75 cm from the middle to end of May. Twenty-five plants per plot were investigated. Anastomosis group 1 (AG-1)^{40,54)} which is the most pathogenic to sorghum and maize, was obtained from sorghum at the National Grassland Research Institute, and incubated for 2 weeks after inoculation onto barley grains. After 2 months of sorghum seeding, the inoculated barley grains were spread on the hill and covered with a thin layer of soil.³³⁾ Resistance to the disease was assessed from the middle of September to early in October. The resistance was expressed by the relative lesion height (RLH); the ratio of the height of lesion on the sheath to that of the flag leaf collar²²⁾:

$$\text{RLH (\%)} = \frac{(\text{height of lesion on the sheath})}{(\text{height of the flag leaf collar})} \times 100$$

Criteria for selection

Promising individuals were selected for resistance to *Rhizoctonia* sheath blight by the field inoculation test from F₂ to F₄ generations. The criterion for the selection was on RLH under 20%. From F₅ to F₈ generations, in addition to RLH by the head-to-row test, the lines were selected for lesion height under 30 cm on the sheath and for superior characters for cultivation. The rate of selection to lines was 25% on the average. Four superior plants were selected from each line and evaluated by inoculation tests in the next year. Consequently, 25 F₈ lines derived from 3 cross combinations were selected as promising lines for the resistance in 1993.

Table 5.1 Breeding resources of resistance to *Rhizoctonia* sheath blight.

Genotype		RLH ^{a)}	Height of the lesion on the sheath (cm)	Total number of F ₂ plants
	M91034	20	20	
	M36001	20	21	
	F6-3A-5	27	24	
	Senkinshiro ^{b)}	73	81	
	Waikai(1) ^{b)}	68	54	
Seed parent	Pollen parent			
F6-3A-5×	Senkinshiro			178
F6-3A-5×	M36001			170
M91034×	Waikai(1)			240

a): RLH: Relative Lesion Height. Values obtained in 1993 and 1994.

b): Susceptible variety and Korean type.

Testing of specific combining ability (SCA)

The SCA was also tested by using 25 F_8 lines derived from 2 combinations. The F_1 lines were crossed between Redbine selection 3048A as seed parent and the F_8 lines as a pollen parent in 1993 and 1994. Redbine selection 3048A, which has cytoplasmic male sterility and a higher combining ability, is the seed parent of Suzuho. The F_8 lines and Suzuho were grown in the same experimental field for comparison. Methods of cultivation, field inoculation test and observation were the same as those for selection.

5.2. Results

From the F_2 to F_5 generations, RLH and the percentage of plants with an RLH under 20% were examined (Fig.5.1). The average RLH in the 3 combinations was about 25% and the variance among generations was small. The percentage of the selected plants with an RLH under 20% decreased with

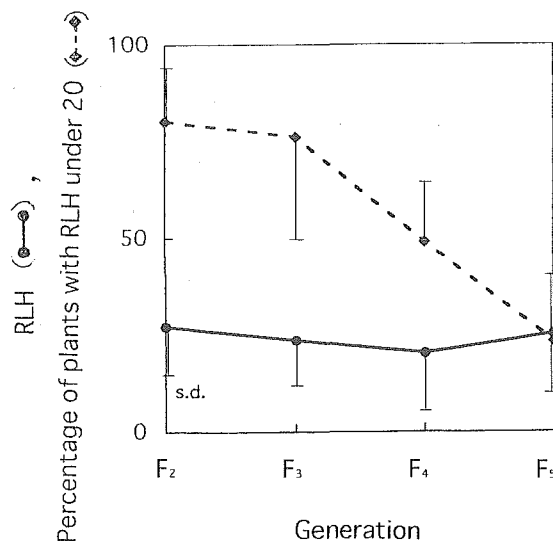


Fig.5.1 Change of RLH from F_2 to F_5 generations. Average RLH in 3 combinations. RLH: Relative lesion height.

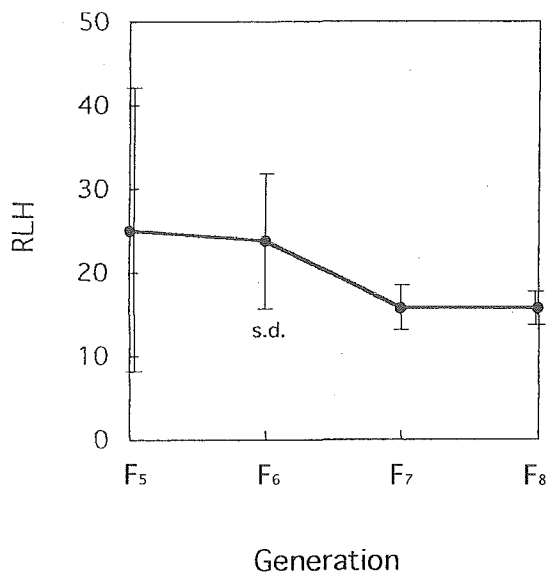


Fig.5.2 Change of RLH from F_5 to F_8 generations. Average RLH in 3 combinations. RLH: Relative lesion height.

advancing generation and showed a steep fall after the F_3 generation.

The average RLH in the 3 combinations decreased from 25% to 15% under the selection by head-to-row test from F_5 to F_8 generations (Fig.5.2). The lines of F_7 and F_8 showed a stronger resistance than variety Suzuho to *Rhizoctonia* sheath blight under field conditions.

The RLH of the selected F_8 lines was lower than that of the parents in F6-3A-5×Senkinshiro or like that in F6-3A-5×M36001 and M91034×Waikai(1) on the average in the results of 1993 and 1994 (Fig.5.3).

The results of the experiment for SCA are shown in Fig.5.4. The distribution of RLH was not shown for M91034×Waikai(1), because the number of lines selected in F_8 was small.

There was no significant difference between the average RLH in F_8 lines derived from F6-3A-5×M36001 and that in the F_1 lines crossed between Redbine selection 3048A (seed parent) and the F_8 lines (pollen parents) (right in Fig.5.4), while there was significantly different at the 5% level in F6-3A-5×Senkinshiro and that in the F_1 lines (left in Fig.5.4). The average for the F_1 lines produced for observation of SCA was significantly higher than that for the F_8 lines derived from F6-3A-5×Senkinshiro. The mean value of the RLH in the F_1 lines developed by using F_8 lines which were derived from F6-3A-5×Senkinshiro and F6-3A-5×M36001 as pollen parents was about 15% lower than that of the commercial variety Suzuho which was derived from the same parent, Redbine selection 3048A. The HL was 45.3 cm and 26.5 cm on the average for 2-years in the F_1 lines developed by using F_8 lines which were derived from F6-3A-5×Senkinshiro and F6-3A-5×M36001 as pollen parents, respectively. In the latter case, the height of the wilting lesion on the sheath was no longer distinguishable from the natural drying-off by aging.

The RLH and the HL were 34% and 54 cm on the average for 2 years in the commercial variety Suzuho which was the F_1 produced by crossing Redbine selection 3048A with Senkinshiro.

5.3. Discussion

The resistance to the disease is commonly expressed by the infected sheath height. On the other hand, RLH is also practically used as an important marker of the resistance to *Rhizoctonia* sheath blight because there is a high positive correlation between RLH and yield loss (See Chapter 3)^{22,24,25}. Since the height of the flag leaf collar varies with the plant type in sorghum, it may be reasonable to express the damage to the plant by the ratio of the infected length to the whole sheath length. KASUGA and GAU²⁷ reported that RLH is also needed for selection in addition to the infected sheath height. In this study, the F_1 lines developed for SCA had higher resistance than the commercial variety, confirming that these two characters are practically useful for screening the resistance to the disease by a field inoculation test.

In the early generation of screening, although the plants with a lower RLH were selected, the ratio of

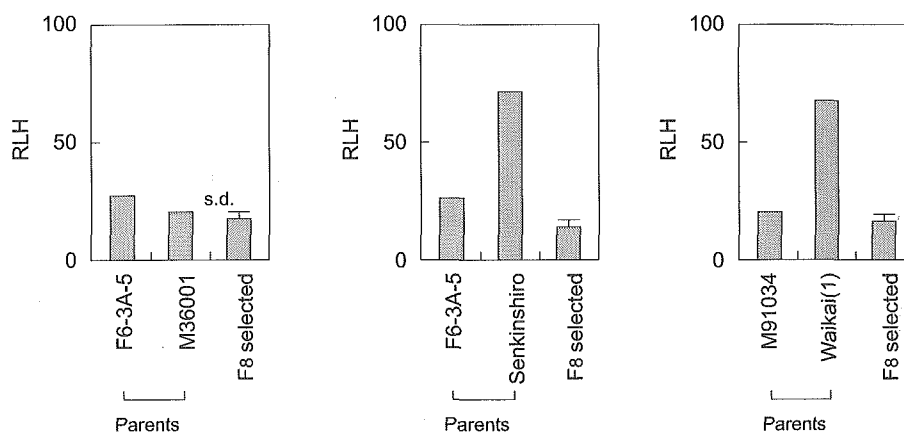


Fig.5.3 Effects of selection on RLH.
RLH : Relative lesion height.

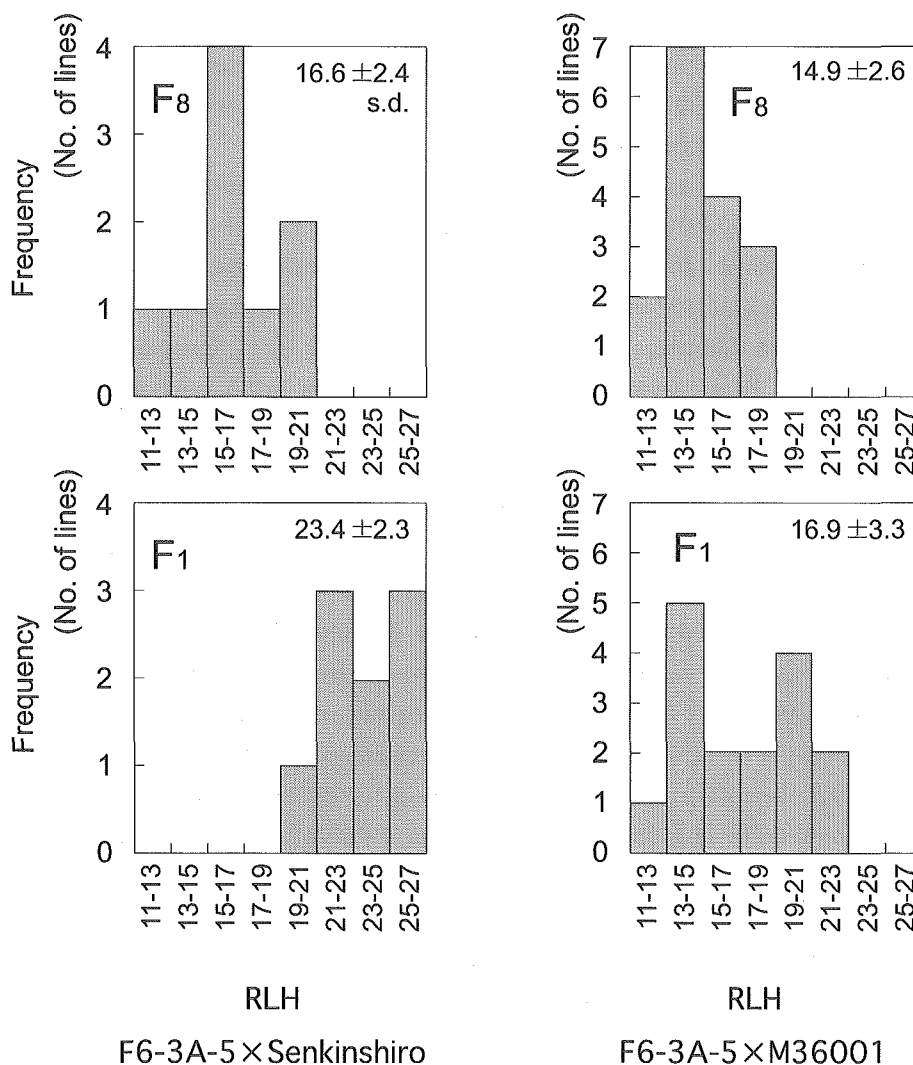


Fig.5.4 Distributions of RLH in F₈ lines selected and the F₁ lines crossed between Redbine selection 3048A as seed parent and F₈ lines as pollen parent (SCA). RLH: Relative lesion height.

the plants with an RLH under 20 was higher than that in a later generation. This may be caused by diminishing heterosis for the resistance to the disease. In a later generation of selection, especially after the F₅ generation, the selection for lines seems to be effective for improving the resistance.

Because most of the modern varieties of sorghum are hybrids, the resistance expressed in the inbred line would be required for practical use. Thus the combining ability is an important character, and I carried out the testing of SCA in this study. KASUGA and OGIWARA²⁶⁾ pointed out that the resistance to the disease was correlated with that of both parents because the average RLH of the parents was closely correlated with that of the F₁ lines. In the inbred lines derived from the F₈ lines of F6-3A-5 × M36001 in this experiment, the resistance to disease of the F₈ lines used was sufficiently expressed in the F₁ lines. In this case, the resistance in F₁ seemed to be improved to the same level as that in the parents.

SHAUG *et al.* (1988)⁴⁸⁾ showed from a diallel analysis that the inheritance of the resistance to sheath blight in sorghum was a quantitative characteristic and the gene actions exhibited additive dominant effects. They also showed that the partial dominant effects and asymmetry of positive and negative effects of genes existed in the resistant system. From their information, the present results and the previous results in this study as shown in Chapter 4, testing of combining ability is obviously needed in the breeding

system for resistance to this disease.

In conclusion, hybrids, which have resistance to *Rhizoctonia* sheath blight, can be developed practically by selecting inbred lines based on the RLH and the HL at the maturing stage in field inoculation tests and by investigating the combining ability of the inbred lines selected. The selection of inbred lines by this method should be useful for the improvement of resistance in sorghum breeding and also may be the most practical way to control the disease without the negative impact to the environment caused by the current use of pesticides.

Now, 20 F_8 lines were selected from the experimental lines by observing degrees of fixation and fertility restoring, and were tested the general combining ability.

5.4. Summary

The effectiveness of a breeding system using a field inoculation test was evaluated for producing hybrids resistant to sorghum sheath blight. F_2 plants were obtained from 3 cross combinations using a resistant line as a seed parent. The selection by a field inoculation test was started for 200 individuals in the F_2 generation of each combination. Anastomosis group 1, which is the most pathogenic to sorghum, was inoculated to barley grains and the grains were spread on the hill and covered with soil, 2 months after the seeding of the test plants. Resistance to the disease was assessed by the relative lesion height (RLH), the ratio of the height of lesion on the sheath to the height of the flag leaf collar at maturity. The plants with an RLH under 20% were selected from F_2 to F_4 . From the F_5 to F_8 generation, the plants with a lesion height under 30 cm on the sheath in addition to RLH under 20% were selected by the head-to-row-testing. The rate of selection was 25% on the average. The RLH of the selected F_8 lines was lower than or the same as that of the parents. The RLH of F_1 lines obtained by the cross between Redbine selection 3048A as a seed parent and F_8 lines as a pollen parent was the same as that of the F_8 lines or slightly higher. The mean value was about 15% lower than that of the commercial variety Suzuho which was derived from the same seed parent, Redbine selection 3048A. Hybrids with resistance to *Rhizoctonia* sheath blight can be developed by selecting inbred lines based on RLH and the height of the lesion on the sheath at maturity in the field and by evaluating the combining ability of the inbred lines selected.

Key word : breeding, inoculation, specific combining ability

Chapter 6. Effects of brown midrib and bloomless genes on the resistance to sheath blight

Sorghum is superior for dry matter productivity under various environmental conditions, due to its wide range of genetic variation. However, early workers pointed out that sorghum was inferior when compared to maize as a forage crop for digestibility and palatability^{8,9,34-36,38}. Recently, efforts have been made to improve these characteristics by introducing brown midrib (*bmr*)^{2,3,5,10,44,53} and bloomless (*bm*)^{37,53} genes in sorghum. These genes increase the digestibility of fiber and palatability as well as the grain contents in whole plants and non-structural carbohydrates in the stover¹⁵. The *bmr* genes suppress lignification of the fiber in sorghum^{6,7,60} and in a similar manner as brown midrib gene *bm*₃ in maize¹⁴. The brown midrib 18 gene of sorghum (*bmr-18*) is notably more effective than other *bmr* genes and *bm* gene in increasing apparent and true digestibility of fiber^{5,17,58,59} and TDN content in whole crop silage¹⁶. KASUGA *et al.* (1999) released a new sorghum variety "Hazuki", which is a homozygous *bmr-18*, and the TDN content in whole crop silage of this variety is about 10% higher than that of commercial varieties not retaining these genes^{17,29,30,50}.

However, there are apprehensions among breeders that these genes cause a decrease in the dry matter yield per unit area and susceptibility to diseases, insects and lodging. However, there have been no reports on the effects of the introduction of these genes to these agronomic traits. In this chapter, to clarify the

effects of *bmr-18* and *bm* genes on the resistance to sheath blight (*Rhizoctonia solani* Kühn) in sorghum. I compared the resistance to sheath blight of the plants for brown midrib 18 (*bmr-18*), for bloomless (*bm*), for brown midrib 18 and bloomless (*bmr-18+bm*) and normal (N) in an F₂ population developed by crossing the double mutant (*bmr-18+bm*) with normal lines.

6.1. Materials and methods

The materials were 335 plants of an F₂ population derived from a cross between an inbred line "F6-3A-5", a homozygote retaining *bmr-18* and *bm* genes as seed parent, and a normal inbred line "74LH3213" not possessing these two recessive alleles as pollen parent.

The field inoculation test was carried out in the nursery of Nagano Animal Industrial Experiment Station in 1999. Barnyard manure at the rate of 10,000 kg per ha and chemical fertilizer N, P₂O₅ and K₂O at the rate of 140, 160 and 120 kg per ha, respectively, were applied. Half of the N fertilizer was applied as ammonium sulfate at the middle of the vegetative growth period. The F₂ plants were sown with an interhill spacing of 8 cm and interrow spacing of 75 cm on May 26. The Strain of *Rhizoctonia solani* AG-1⁵⁴ which was the most pathogenic to sorghum and maize was obtained from sorghum at the National Grassland Research Institute, were inoculated onto barley grains and incubated for two weeks. The grains were spread on the hills of the nursery on July 21, and covered with a thin layer of soil³³.

Resistance to the disease was evaluated on September 12. The height of lesion on the sheath (HL) and the height of flag-leaf collar (HF) were determined. The relative lesion height (RLH)²⁸ was calculated using the following equation as an indicator of the resistance to the disease and the degree of damage.

$$RLH = \frac{(\text{height of lesion on the sheath, HL})}{(\text{height of the flag-leaf collar, HF})} \times 100 (\%)$$

On September 12, 1999, the plants carrying alleles of for brown midrib 18 (*bmr-18*), for bloomless (*bm*), for brown midrib 18 and bloomless (*bmr-18+bm*) were identified by the color of their leaf midrib and by the wax and color on the surface of leaf sheath.

6.2. Results

The 335 plants in the F₂ population were classified according to their phenotype. 208 plants of normal type (N), 65 plants of brown midrib type (*bmr-18*), 47 plants of bloomless type (*bm*) and 15 plants of brown midrib and bloomless (*bmr-18+bm*) type were identified. The mean values of HL, HF and RLH representing each phenotype are shown in Table 6.1. The analysis of variance of RLH and HL was done using natural logarithmic transformation, because the frequency distribution of RLH and HL did not fit a normal distribution (Fig.6.1, Fig.6.2). However, the frequency distribution of HF fitted a normal distribution (Fig.6.3).

In N and *bmr-18* types, the mean value of RLH was about 30% and the frequency distribution was

Table 6.1 Means of relative lesion height (RLH), height of lesion on the sheath (HL) and height of flag leaf (HF) in each phenotype.

Phenotype	RLH	HL	HF	n*
N	29.6 a	41.7 A	145.6 a	208
<i>bmr-18</i>	32.8 a	42.8 A	133.6 b	65
<i>bm</i>	20.5 b	27.8 B	139.3 ab	47
<i>bmr-18+bm</i>	20.5 b	24.9 B	129.3 b	15

n* : number of plants investigated.

Means in each column followed by the same letter are not significantly different. A and B at p<0.01, a and b at p<0.05.

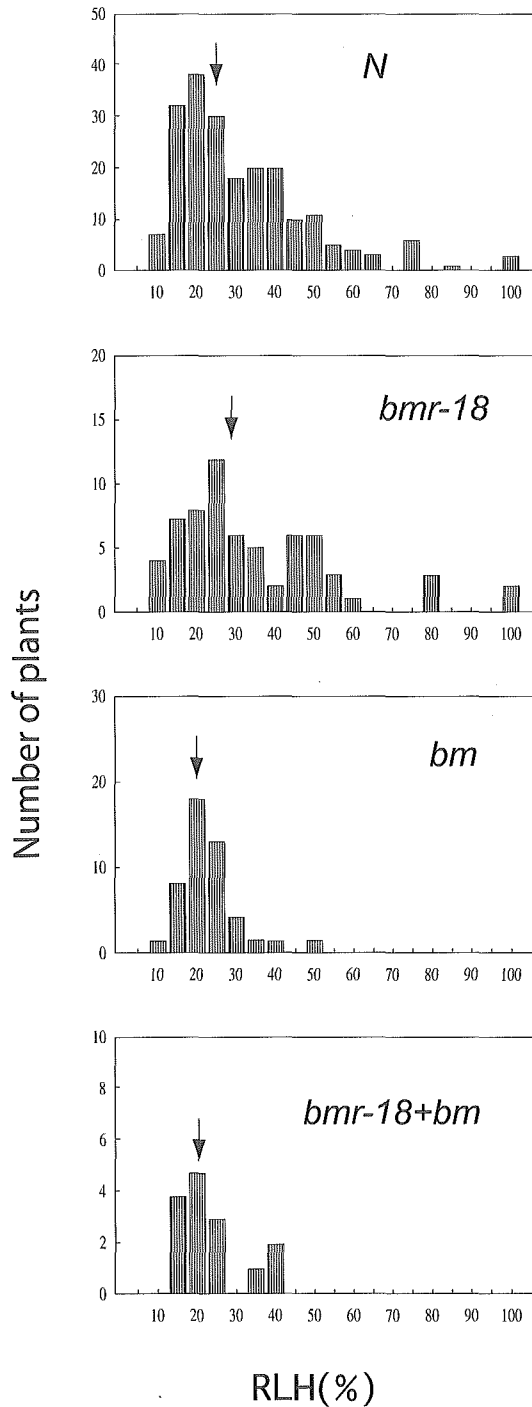


Fig.6.1 Distribution of relative lesion height (RLH).
▼ : mean.

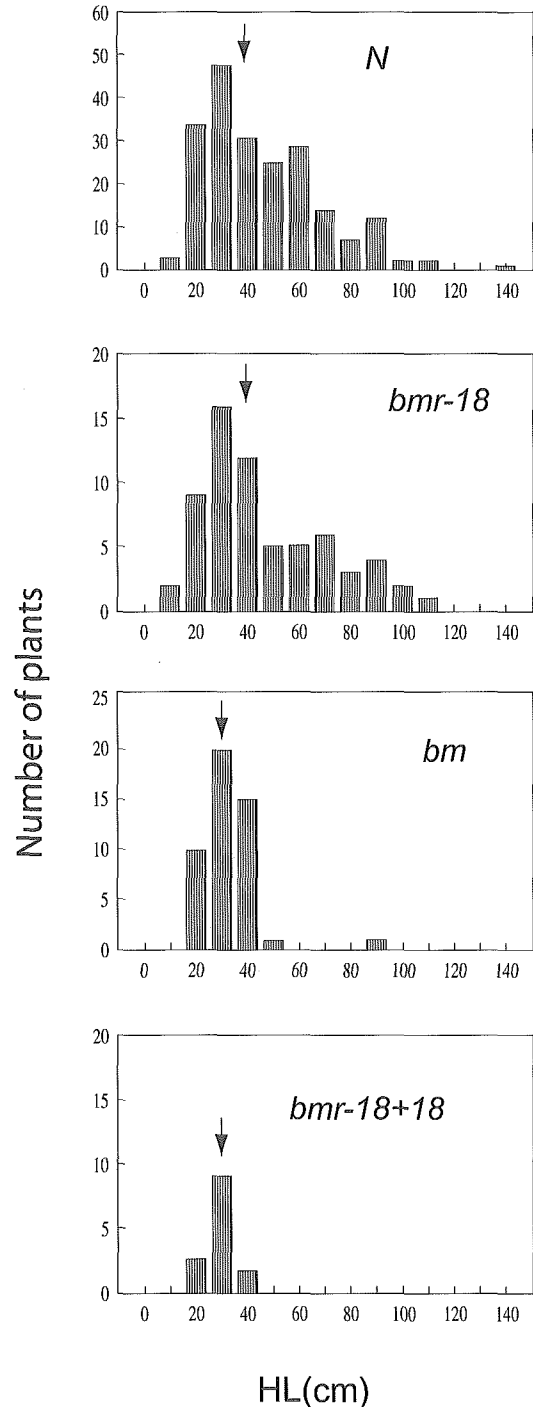


Fig.6.2 Distribution of height of lesion on the sheath (HL).
▼ : mean.

ranged from 6.4% to 100%. However, in *bm* and *bmr-18+bm* types, the mean value of RLH was 20.5%, which was lower than that in *N* and *bmr* types. In addition, the range of the frequency distribution in *bm* and *bmr-18+bm* types was narrower than in *N* and *bmr-18* types (Fig.6.1).

The frequency distribution pattern of HL was similar to that of RLH. The mode of the frequency distribution of HL was about 30 cm in all types. However, the ranges of HL in the *bm* and *bmr-18+bm* type were narrower than *N* and *bmr-18* types (Fig.6.2). The mean values of HL were 27.8 and 24.9 cm in *bm* and *bmr-18+bm* respectively, which were significantly lower than in *N* and *bmr-18* types (Table 6.1).

The frequency distribution of HF was a normal distribution in all types (Fig.6.3), and the mean values

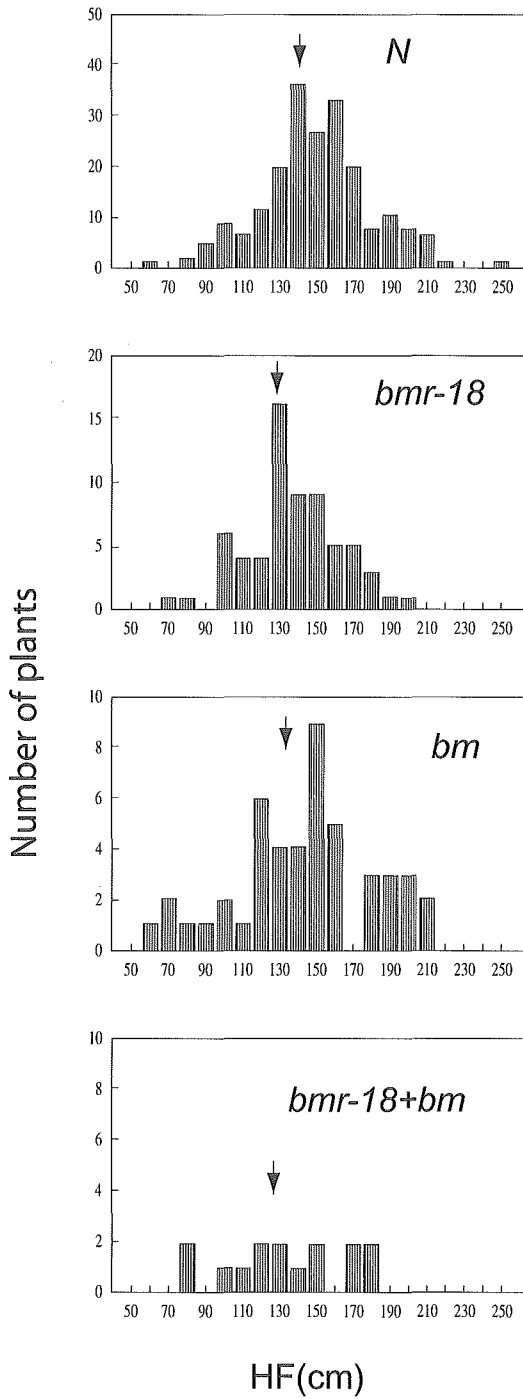


Fig.6.3 Distribution of height of flag leaf (HF).

▼ : mean.

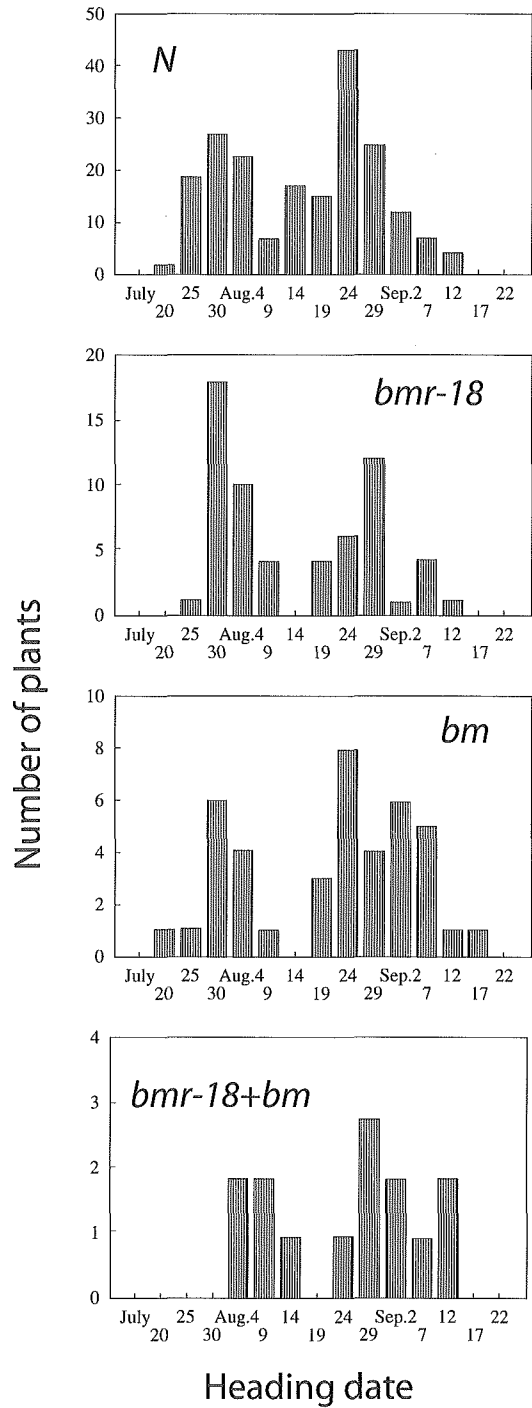


Fig.6.4 Distribution of heading date.

of HL were 133.6 and 129.3 cm in bmr and bmr-18+bm respectively. These values were significantly lower than in N types (Table 6.1).

Fig.6.4 shows the distribution of heading date in each phenotype. The heading date of several plants in each phenotype couldn't be confirmed because of lodging and insects. However, all types seem to have two peaks in the frequency distribution and the peaks occurred in late July and late August for each phenotype. In addition, the mean values of heading date in bm and bmr-18+bm types was later than the N and bmr-18 types (Table 6.2).

Table 6.2 Means of heading date in each phenotype.

Phenotype	Heading date	n*
N	Aug. 15 a	201
bmr-18	Aug. 12 a	61
bm	Aug. 19 b	41
bmr-18+bm	Aug. 22 b	14

n* : number of plants investigated.

Means in each column followed by the same letter are not significantly different at $p < 0.05$.

6.3. Discussion

In a field investigation testing the resistance to sheath blight in sorghum, the breeder needs to examine the morphology and behavior of each genetic line to precisely decide the degree of resistance found each line²⁸⁾. If each plant of the F₂ population exhibits a different morphology and behavior, the resistance of each plant may not be compared directly with other plants.

HF in the N type was taller than that in the bmr-18 type and bmr-18+bm type. The difference among the 4 phenotypes in their HF was about 16 cm. However, the difference of their HF in the F₂ population was relatively small in the level of variation found in sorghum. Therefore, it is considered possible to compare RLH and HL of each type without considering the effect of HF.

In addition to the HF effect, maturity may affect the result of resistance to sheath blight in the field inoculation test²⁸⁾. Therefore, the heading time should be compared among these types before the comparison of resistance to sheath blight. In plants used in this experiment, the range of heading date ranged from late July to mid September. The difference among the 4 phenotypes in heading date was about 10 days. However, the patterns of the distribution of heading date were similar in the 4 phenotypes. Therefore, it is considered that the effect of maturity on RLH and HL is negligible in this population.

It is concluded that the effects of *bmr-18* and *bm* genes on the resistance to sheath blight can be evaluated by a simple comparison among 4 phenotypes without considering the effects of HF and maturity.

The *bmr-18* and *bm* genes, which improve the digestibility of cell wall and nutritive value of sorghum, have been considered to have negative effects on the resistances to diseases, lodging and other agronomic traits. However, RLH and HL in *bm* and *bmr-18+bm* types were significantly lower than those in the N and *bmr-18* types. In addition, there was no significant difference between the *bmr-18* type and N Type in the HL and RLH (Table 6.1). These data suggest that *bm* gene increases the resistance to sheath blight, and that *bmr-18* gene have no negative effects on the resistances to sheath blight.

These results suggest that a new sorghum variety which has resistance to sheath blight and higher nutritive value can be developed by using the *bmr-18* and *bm* alleles. Recently, inbred lines and hybrids have been developed using both characteristics.

6.4. Summary

Effects of brown midrib (*bmr-18*) and bloomless (*bm*) genes on the resistance to sheath blight were investigated in a field inoculation test by using an F₂ population of the cross between "F6-3A-5" (*bmr-18/bmr-18 ; bm/bm*) and "74LH3213" (*Bmr-18/Bmr-18 ; Bm/Bm*). The plants in the F₂ population were classified according to their phenotypes : normal (N), brown midrib (*bmr-18*), bloomless (*bm*) and brown midrib and bloomless (*bmr-18+bm*) types. There were no remarkable differences among these types in the height of flag-leaf collar (HF), heading date, which may affect the result of resistance to sheath blight in the inoculation test. However, the height of lesion (HL) and relative to the height of HF (RLH) in the *bm*

and *bmr-18+bm* types were significantly shorter than those in the N and *bmr-18* types. In addition, there was no significant difference between *bmr-18* type and N Type in the HL and RLH data.

These results suggest that the *bm* gene increases the resistance to sheath blight, and that *bmr-18* gene have no negative effects on the resistances to sheath blight. Furthermore, varieties which are resistant to sheath blight and also possess higher nutritive values can be developed by using *bm* and *bmr-18* genes.

Key word : bloomless, brown midrib

Chapter 7. Summary and conclusion

In this study, I clarified the criteria for the selection of the resistance to sheath blight by the examinations for the varietal difference and the yield loss by the infection in sorghum. The effectiveness of a breeding system using the field inoculation test was evaluated by developing hybrids resistance to sheath blight. Furthermore, the effects of brown midrib (*bmr-18*) and bloomless (*bm*) genes on the resistance to sheath blight were investigated.

These results suggested that the breeding system with field inoculation test could develop the new sorghum varieties, which have resistance to sheath blight and high forage quality.

The results obtained in this study are as follows.

1. In order to search for genetic resources of resistance to sorghum sheath blight, the varietal differences in the resistance were examined by a field inoculation test in 1984 and 1985.

The materials used were 72 varieties and lines, which were classified into grain, dual purpose (F_1) and sorgo types. Barley grains were inoculated with anastomosis group 1 (AG-1), which was the most pathogenic to sorghum, and the grains were spread on the hill and covered with soil two months after the seeding of the test plants. Resistance to the disease was evaluated by the relative lesion height (RLH), which is the ratio of the height of the lesion on the sheath (HL) to the height of the flag leaf collar (HF) at maturity.

The varietal differences of HL, HF and RLH were similar in both years. The relationship between HL and RLH in each of the three groups, grain type including male sterile lines, dual-purpose type including Korean type and sorgo type including Indian type, was regressed to a linear equation. More highly resistant resources were found in every type, and the common criteria of the resistance were considered to be an RLH of less than 20% and HL of less than 20 cm.

2. To clarify the selection criterion of the resistance to sheath blight in Sorghum, the field inoculation test was carried out in 1985.

The materials used were 72 varieties and lines, which were classified into grain types, dual-purpose types and sorgo types. Resistance to the disease was evaluated by RLH at maturity. Yield loss by the disease was evaluated by the relative dry matter weight (RDM), which is the ratio of the dry matter weight of inoculated plants to non-inoculated plants.

There were varietal differences in the yield loss in 72 varieties by sheath blight. The relationship between RLH and RDM was fitted well a reciprocal model. The reciprocal model for RDM based on the relationship between RLH (x) and RDM (y), was as follows : $1/y = 0.01 + 0.0002(x - 14.2)$.

The common criteria of the resistance, which are considered to be RLH of less than 20% and HL of less than 20 cm from the relationship between RLH and HL, is effective from the viewpoint of the relationship between RLH and RDM.

3. Diallel analysis of the resistance to sheath blight was performed using 5×5 reciprocal crosses of lines belonging to the grain type by a field inoculation test to clarify the inheritance of the resistance to sheath blight.

The mean additive effect, mean dominance and remaining dominance appearing in a specific combination were significant for RLH at the 1 % level of probability. The results of the analysis for RLH could

fit the additive and dominant model without an epistasis effect. The heritability was high in a narrow (0.773) and broad sense (0.935). The additive effect for HL was significant at the 1 % level, while the dominant effect was not. The results of the analysis for HF did not fit the additive and dominant model. There was a significant correlation between the average RLH of better resistant and mid parent $((BP+MP)/2)$ and that of the hybrids ($r=0.942, p<0.001$). The additive effect was stronger than the dominant effect for RLH. Therefore, the selection for RLH in an early generation is effective to develop inbred lines rapidly.

4. The effectiveness of a breeding system using a field inoculation test was evaluated by developing hybrids resistant to sheath blight. F_2 plants were obtained from 3 cross combinations using a resistant line as a seed parent. The selection by a field inoculation test was started for about 200 individuals in the F_2 generation of each combination. Resistance to the disease was assessed by RLH at maturity. The plants with an RLH under 20% were selected from F_2 to F_4 . From the F_5 to F_8 generation, the plants with HL under 30 cm in addition to RLH under 20% were selected by the head-to-row-testing. The rate of selection was 25% on the average.

The RLH of the selected F_8 lines was lower than or the same as that of the parents. The RLH of F_1 lines obtained by the cross between Redbine selection 3048A as a seed parent and F_8 lines as a pollen parent was the same as that of the F_8 lines or slightly higher. The mean value was about 15% lower than that of the commercial variety Suzuho which was derived from the same seed parent, Redbine selection 3048A. Hybrids with resistance to *Rhizoctonia* sheath blight can be developed by selecting inbred lines based on RLH and HL at maturity in the field and by evaluating the combining ability of the inbred lines selected.

5. Effects of brown midrib (*bmr-18*) and bloomless (*bm*) genes on the resistance to sheath blight were investigated in a field inoculation test by using an F_2 population of the cross between F6-3A-5 (*bmr-18/bmr-18;bm/bm*) and 74LH3213 (*Bmr-18/Bmr-18 ; Bm/Bm*).

The plants in the F_2 population were classified according to their phenotypes : normal (N), brown midrib (*bmr-18*), bloomless (*bm*) and brown midrib and bloomless (*bmr-18+bm*) types. There were no marked differences among these types in HF, heading date, which may affect the result of resistance to sheath blight in the inoculation test. However, the HL, RLH in the *bm* and *bmr-18+bm* types were significantly shorter than those in the N and *bmr-18* types. In addition, there was no significant difference between *bmr-18* type and N Type in the HL and RLH data.

These results suggest that the *bm* gene increases the resistance to sheath blight, and that the *bmr-18* gene has no negative effects on the resistance to sheath blight. Furthermore, varieties which are resistant to sheath blight and also possess higher nutritive values can be developed by using *bm* and *bmr-18* genes.

From these results, 2 new varieties, "Hazuki" and "Akidachi" could be released in 1998 and 2001. These varieties have homozygote for *bmr-18* and sufficiently higher resistance to sheath blight under field condition.

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* : In English

** : In Japanese with English summary

*** : In Japanese only. Translated title by the present authors

**** : In Chinese with English summary

ソルガムの紋枯病 (*Rhizoctonia solani* Kühn) 抵抗性品種の育成に関する研究

春日 重光

信州大学農学部附属アルプス圏フィールド科学教育研究センター

Japanese Summary

第1章 緒論

1955年ごろからの経済発展にともない、農業の大規模専業化が急激に進められた結果、農業経営は稲作、野菜、果樹などの耕種農家と畜産農家に専業化した。農業におけるこのような選択的拡大は、従来までの小規模・有畜農業に比べ、農業所得の増加をもたらした。しかし、大規模専業化により生産地の局在化が進むと同時に、生産物の過剰と生産調整あるいは耕種農家における地力低下と畜産農家における糞尿処理問題など多くの弊害が発生してきた。

現代の少品目大量生産・連作型の農業経営においては、持続的な生産のための地力維持が極めて重要になっている。しかし、耕種農家における有機質資材の不足と畜産農家における糞尿処理は、両者間で順調に解決されない事例が多く、その結果、畜産農家は糞尿処理に多くの経費を支出している。

一方、畜産経営における極端な規模拡大は、穀物のみならず、粗飼料についても輸入依存度を高め、我が国の国内産飼料自給率は年々減少し、畜産経営の基盤を不安定にしている。

こうした状況の中で、畜産物の安全性や糞尿に関わる環境問題あるいは飼料自給率を考えると、吸肥力、乾物生産性及び飼料価値に優れた飼料作物栽培の振興は、今後の我が国の畜産経営に不可欠である。

著者は、こうした視点から畜産経営に対応するため、吸肥力、耐干・耐湿性などの環境ストレス耐性、乾物生産性及び再生力などに優れ、作物として極めて大きな変異を持つソルガムの畜産への利用に着目し、1982年から飼料用ソルガムの育種に従事してきた。

一方、飼料用ソルガムは、コメの生産調整による減反政策が進められるなかで、水田転換畑への作付けの比率が増加してきた。しかし、家畜糞尿が多投された水田転換畑においては、夏季の高温・多湿条件下で、紋枯病の発生が多発した。この紋枯病は、下葉の枯上り、幼苗期の立ち枯れ、出穂の遅延や最終的には罹病株の倒伏によって、ソルガムに大きな減収をもたらす場合が多く、減反政策の強化や連作

などにより被害の増加が懸念された。

紋枯病菌の *Rhizoctonia solani* Kühn は、極めて宿主範囲の広い菌でイネやトウモロコシの紋枯病をはじめ、野菜類についても多くの病害が報告されている。*Rhizoctonia solani* Kühn はその菌糸の融合性から、現在11の菌糸融合群として分類され、宿主によって病原性のある菌糸融合群が明らかになっている。我が国における、ソルガムの紋枯病については、これらの菌糸融合群のうち AG-1 及び AG-2 による発病が確認されている。この AG-1 菌糸融合群はイネやトウモロコシの紋枯病と同じ菌糸融合群であり、飼料作物畑、水田転換畑を問わず土壤中に菌核の形で存在する。しかし、飼料としての安全性や生産コストの面から、イネなどのような薬剤防除がほとんど不可能な飼料用ソルガムでは、その防除は極めて困難である。

こうした背景のなかで、安定したソルガム栽培を行うためには、紋枯病について、発病しても減収や品質低下が顕著にならないようにするための圃場抵抗性の改良が最も有効だと考えられる。

一方、飼料用ソルガムの育種目標として従来からあげられている多収性、耐倒伏性および病害虫抵抗性などに加え、近年は飼料としての消化性や品質の改良が重要視されている。こうした品質改良の手段として *bmr* (brown midrib: 褐色中肋) などの高消化性遺伝子の利用が1970年代から試みられてきたが、この遺伝子を持つものは、耐倒伏性や耐病性が劣るというマイナスイメージから、これの実用化が遅れている。しかし、近年、家畜の飼養方法や栽培・利用法などの多様化が急速に進みつつあり、高消化性遺伝子を持つ実用品種の開発が急務である。このため、*bmr* などの高消化性遺伝子とソルガムの紋枯病抵抗性との関係を検討することは、高品質飼料用ソルガムの育種を進める上で極めて重要である。

著者は以上のようなソルガム育種を取り巻く状況から、ソルガムの紋枯病について AG-1 菌糸融合群

に対する圃場抵抗性の育種改良を目的として、紋枯病による圃場抵抗性の品種間差異の検討と紋枯病による被害程度の検討を行い、圃場検定における選抜基準の設定とその選抜基準を用いた抵抗性品種育成の可能性を検討した。また、抵抗性の遺伝的機作とそれに基づく選抜方法を検討するため、ダイヤレル交配による抵抗性の解析を試みた。さらに、高品質育種のために導入した *bmr* などの高消化性遺伝子が紋枯病抵抗性に及ぼす影響を検討し、紋枯病抵抗性で品質に優れた母本の育成の可能性について検

討した。

これらの結果、育種現場における紋枯病抵抗性の検定と評価法、抵抗性母本の育成方法および高消化性遺伝子を導入した紋枯病抵抗性母本育成の手法について成果を得た。そして、これらの知見をもとに高消化性遺伝子 *bmr-18* を持ち、実用レベルの紋枯病抵抗性を持つ新品種「葉月」(1998年)、「秋立」(2001年)を育成し、農林登録を行い普及に移したので、ここに研究成果の概要を報告する。

第2章 ソルガムの紋枯病圃場抵抗性に関する品種間差異の検討

ソルガム紋枯病抵抗性品種の育種素材を見出すため、抵抗性の品種間差異を圃場接種検定法によって調査した。用いた材料は子実型、兼用型及びソルゴー型に属する合計72の品種・系統である。これらの中には食用に選抜されたインド型品種、高粱型品種及び雄性不稔系統も含めた。圃場試験は1984年と1985年の2年間行った。ソルガムに高い病原性を示す紋枯病の菌糸融合群 AG-1 に属する病原菌を、大麦穀粒で培養した後、播種から2か月後のソルガムに半覆土法によって1個体当たり4gを接種した。その後、成熟期に葉鞘高(HF)と病斑高(HL)を測定し、圃場抵抗性の指標として病斑高率(RLH; $HL \div HF \times 100, \%$)を求めた。年次が異なっても HL, HF および RLH の品種間差異は同

じ傾向を示し、年次間の相関係数はいずれも0.1%水準で有意であり、高い再現性が認められた。病気の進展を示す HL と被害程度を示す RLH の関係は一次回帰式に良く適合した ($p < 0.001$)。それらの回帰式はタイプにより異なり、子実型と雄性不稔系統群、兼用型一代雑種と高粱型品種群、ソルゴー型とインド型品種群の3群に区別された。草丈を示す HF と RLH の回帰式も同様の3グループに区別された。子実型品種群は草丈が同じでも RLH に大きな品種間差がみられた。どのタイプも紋枯病抵抗性を持つ優れた遺伝資源を含み、それらに共通する抵抗性判定基準としては RLH が20%以下でかつ HL では20cm以下が有効と考えられた。

第3章 紋枯病によるソルガムの減収程度と抵抗性判定指標の設定

ソルガム紋枯病の抵抗性育種素材を選抜するため、紋枯病による減収程度と紋枯病抵抗性の関係について1985年に検討した。供試した材料は子実型、兼用型及びソルゴー型に属する合計72の品種・系統である。1品種・系統当たり15個体について、AG-1 菌糸融合群に属する病原菌を、播種から2か月後に半覆土法によって接種した。罹病調査は成熟期に行い、病原菌を接種した個体について HF と HL を測定し、圃場抵抗性の指標として RLH を求めた。また、1品種・系統当たり各々5個体の病原菌接種株および非接種株の乾物重を測定し、減収程度の指標として相対乾物重(RDM; $\text{接種株の乾物重} \div \text{非接種の乾物重} \times 100, \%$)を求めた。

病原菌接種による発病は順調で、接種株は非接種株より草丈、稈長及び葉鞘高が7~8cm程度減少した。供試品種・系統の HL は9cm~70cm, RLH は7.4%~100%, RDM は22%~100%で、紋枯病の罹病程度及び減収程度に大きな品種・系統間差が認められた。RLH(x)とRDM(y)の関係は $1/y = 0.01 + 0.0002(x - 14.2)$ ($r = 0.841$) の逆数モデルに適合し、減収が始まる RLH は14.2%であった。HL と RLH の関係(第2章参照)及び RLH と RDM の関係から、紋枯病抵抗性系統の圃場における接種検定の選抜基準としては、RLH が20%以下でかつ HL では20cm以下が有効と考えられた。

第4章 ソルガム紋枯病抵抗性のダイヤレル分析

ソルガム紋枯病の圃場抵抗性の遺伝を調べるためにフル・ダイヤレル分析を実施した。用いた材料は抵抗性が大きく異なる子実型5系統で、これらに

AG-1 菌糸融合群に属する病原菌を播種から2か月後に半覆土法によって接種した。その後、成熟期に HF と HL を測定し、RLH を求めた。その結果、

RLHの相加・優性効果は1%で有意であり、エピスタシスのない相加・優性モデルに適合した。狭義及び広義の遺伝率は、各々0.773と0.935であった。HLでは相加効果は有意であったが、優性効果は認められなかった。HFは相加・優性モデルに適合し

なかった。RLHに関して抵抗性親と中間親の平均値と一代雑種の間には高い正の相関が認められた($r=0.942$, $p<0.001$)。RLHの相加効果は優性効果よりも大きかったため、選抜初期世代からの選抜効果は高いと考えられた。

第5章 ソルガムの紋枯病圃場抵抗性に関する選抜とその改良

ソルガム紋枯病圃場抵抗性について、圃場接種検定をもとにした選抜の有効性を検討した。抵抗性の強い品種を種子親にし、3組合せの交配を実施した。各組合せとも約200個体のF₂集団から紋枯病菌接種による個体選抜を開始した。AG-1菌糸融合群に属する病原菌を播種から2か月後に半覆土法によって全個体に接種した。そして、成熟期にRLHを測定し、20%以下の個体のみを選抜し、選抜個体の等量混合種子により世代を進め、F₄世代まで繰り返した。さらに、F₅世代からF₈世代までは1穂1列法によりRLHに加えてHLが30cm以下で劣悪形質の無い系統を選抜した。このさいの選抜率は約25%とした。

最終的に得られたF₈系統のRLHは、両親のうちの強い系統と比べて同等がそれ以下となり、抵抗性の強い実用的な母本が育成できた。さらに、市販品種の「スズホ」と同じ種子親「Redbine selection 3048A」を用い、育成したF₈系統を花粉親にした一代雑種系統のRLHは、育成したF₈系統のRLHと同等かやや高く、「スズホ」よりも平均で約15%低かった。これらのことから、圃場で病原菌を接種してRLHとHLに基づいて選抜し、さらに組合せ能力検定することは、紋枯病抵抗性一代雑種品種を育成するのに有効と考えられた。

第6章 ソルガムの高消化性遺伝子が紋枯病圃場抵抗性に及ぼす影響

高消化性遺伝子である褐色中肋遺伝子(*bmr-18*)及び無白粉形茎遺伝子(*bm*)がソルガム紋枯病圃場抵抗性に及ぼす影響を明らかにするため、これら2つの高消化性遺伝子に関するホモ接合体である自殖系統「F6-3A-5」を種子親に、これらの遺伝子を持たない自殖系統「74LH3213」を花粉親にした組合せのF₂世代の集団を用い、圃場での接種検定によって、表現型別の紋枯病抵抗性を比較した。供試個体は表現型により、高消化性遺伝子の発現なし(N)、褐色中肋のみ発現(*bmr-18*)、無白粉形

茎のみ発現(*bm*)、褐色中肋と無白粉形茎の両方を発現(*bmr-18+bm*)の4タイプに分類した。葉鞘高と出穂期は抵抗性の判定結果に影響する形質であるが、4タイプの間で顕著な差は無かった。*bm*のRLHとHLは、N及び*bmr-18*よりも低く、紋枯病抵抗性の改良効果があることが推察された。また、*bmr-18*とNでは、RLHとHLの間には有意な差はなかった。これらのことから、*bm*及び*bmr-18*遺伝子の利用によって紋枯病抵抗性と高消化性をあわせ持つ品種の育成が可能と考えられた。

第7章 総括及び結論

本論文は、ソルガムの紋枯病圃場抵抗性の改良を目的に、ソルガム品種・系統の持つ紋枯病抵抗性の差異を把握し、抵抗性系統の選抜・育成方法の検討とその遺伝的解析の成果及びソルガムの高消化性遺伝子と紋枯病抵抗性の関係について検討した成果を述べたものである。

得られた成果の概要は次のとおりである。

1. ソルガム紋枯病抵抗性に関する育種素材を見出すため、2か年にわたって子実型、兼用型およびソルゴー型に属する72品種・系統を用い圃場接種検定によって成熟期の品種間差異を検討した。罹病程度は、葉鞘高(HF)と病斑高(HL)を測定し、病斑高率(RLH; $HL \div HF \times 100$, %)を求めて比

較した。罹病程度の品種間差異は、年次による再現性が認められ、HLとRLHの関係は一回帰式に良く適合した($p<0.001$)。それらの回帰式はソルガムのタイプによって異なり、子実型と雄性不稔系統群、兼用型一代雑種と高粱型品種群、ソルゴー型とインド型品種群の3グループに区別されたが、各グループともに枯病抵抗性を持った遺伝資源を含み、抵抗性品種の選抜・育成が可能であると考えられた。また、これら3グループのHLとRLHの一回帰式の交点はRLHが20%、HLが20cm程度であり、共通する抵抗性判定基準としてはRLHが20%以下でかつHLでは20cm以下が有効と考えられた。

2. 紋枯病によるソルガムの減収程度と紋枯病抵

抗性の関係について、子実型、兼用型、ソルゴー型に属する72品種・系統を供試して検討した。圃場抵抗性の指標としてRLH、減収程度の指標として病原菌非接種株に対する病原菌接種株の相対乾物重(RDM, %)を求めた。紋枯病の罹病程度および減収程度には大きな変異が認められ、RLH(x)とRDM(y)の関係は $1/y=0.01+0.0002(x-14.2)$ ($R^2=0.742$)の逆数モデルに適合した。HLとRLHの関係及びRLHとRDMの関係から、紋枯病抵抗性系統の圃場における接種検定の選抜基準としては、RLHが20%以下でかつHLが20cm以下が適当であると考えられた。

3. ソルガム紋枯病の圃場抵抗性が大きく異なる子実型5系統を用い、フル・ダイアレル分析を実施した。その結果、RLHの相加・優性効果は1%で有意であり、エピスタシスのない相加・優性モデルに適合した。狭義及び広義の遺伝率は、各々0.773と0.935であった。HLでは相加効果は有意であったが、優性効果は認められなかった。HFは相加・優性モデルに適合しなかった。RLHに関して抵抗性親と中間親の平均値と一代雑種の間には高い正の相関が認められ($r=0.942$, $p<0.001$)、RLHの相加効果は優性効果よりも大きかったので、選抜初期世代からの選抜効果は高いと考えられた。

4. 圃場接種検定をもとに、紋枯病圃場抵抗性について選抜の有効性を検討した。抵抗性の強い品種を片親に持つ3組合せの交配を行い、各組合せとも約200個体のF₂世代の集団から紋枯病菌接種による個体選抜を開始した。選抜は、収穫期のRLHにより行い、20%以下の個体のみを選抜し、選抜個体の等量混合種子により世代を進め、F₄世代まで繰り返した。さらに、F₅世代からF₈世代までは1穂1列

法によりRLHに加えてHLが30cm以下で劣悪形質のない系統を選抜した。系統の選抜率は約25%とした。最終的に得られたF₈系統のRLHは両親のうちの強い系統と比べて同等がそれ以下となり、抵抗性の強い実用的な母本が育成できた。さらに、市販品種の「スズホ」と同じ種子親を用い、それらの育成系統を花粉親にした一代雑種系統のRLHは、育成したF₈系統の病斑高率と同等かやや高く、「スズホ」よりも平均で約15%低かった。これらのことから、RLHとHLを指標にした圃場接種検定は、紋枯病抵抗性品種の育成に有効と考えられた。

5. 高消化性遺伝子である褐色中肋遺伝子(*bmr-18*)及び無白粉形茎遺伝子(*bm*)が、ソルガムの紋枯病圃場抵抗性に及ぼす影響を明らかにするため、これら2種の高消化性遺伝子に関するホモ接合体である自殖系統「F6-3A-5」を種子親に、これらの遺伝子を持たない自殖系統「74LH3213」を花粉親にした組合せのF₂世代の集団を用い、高消化性遺伝子の発現なし(N)、褐色中肋のみ発現(*bmr-18*)、無白粉形茎のみ発現(*bm*)、褐色中肋と無白粉形茎の両方を発現(*bmr-18+bm*)の4タイプの表現型間で紋枯病抵抗性を比較した。その結果、*bm*遺伝子には抵抗性の改良効果があることが推察された。また、*bmr-18*遺伝子による抵抗性の低下は認められなかったことから、紋枯病抵抗性と高消化性をあわせ持つ品種の育成が可能であることが明らかとなった。

6. 以上の成果を活用して、著者らは、高消化性遺伝子*bmr-18*を持ち、紋枯病抵抗性について実用レベルにある新品種「葉月」及び「秋立」の農林登録を行い、普及に移した。

キーワード：ソルガム、紋枯病、接種検定、減収程度、ダイアレル分析、高消化性遺伝子