

Chapter 1 Preface

1.1 History of researches regarding as cold-weather damage and breeding in soybeans.

Hokkaido, which is the largest soybean production area in Japan, is located in northern Japan. Soybean cultivation in Hokkaido started in around 1870. In the beginning, soybean was grown only in the southern part of Hokkaido. Then, the cultivation area was expanded from the southern to the eastern and the northern parts of Hokkaido. This also means that the soybean production of Hokkaido expanded into a cool region. Although the eastern part of Hokkaido is the main soybean producing region, the soybean production in the part has been often damaged from low temperature during the soybean growth.

Cold-weather damage in Hokkaido is caused mainly by three factors (Yamamoto and Narikawa, 1966): 1) Poor growth due to low temperature at the early stage of growth, 2) flower abscission and pod setting failure due to low temperature prior to and during the flowering stage, 3) insufficient grain filling due to low temperature in the pod filling stage. The damage temperature lies 13-17 °C in poor growth (Sanbuichi, 1979), 15-18 °C in flower and pod abscission (Toriyama and Toyokawa, 1957; Saito et al., 1970), 12-15 °C in insufficient grain filling (Ushirogi et al., 1973), respectively. As described later, the frequency of the years with damage by poor growth was the highest in Hokkaido. Therefore, the Tokachi Agricultural Experiment Station started soybean breeding for poor growth due to low temperature at the early stage of growth in 1964 as a beginning. Tsuchiya et al. (1976) and Sanbuichi (1979) reported that growth vigor under low temperature conditions is correlated with cold-weather tolerance in the early stage of growth. Superior growth vigor soybeans were selected in fields where temperature during the growth is 1 to 2 °C lower than the average air temperature. As a result, Kitamusume (Saito et al., 1969) and Toyohomare (Yumoto et al., 1995) were released.

Breeding and researches relating poor growth due to low temperature at the early stage of growth have been also conducted in Switzerland (Schmid and Keller, 1980) and Russia (Sichkar, 1988). There is a similarity in tolerant soybeans between above two countries and Hokkaido; tolerant soybeans show superior growth vigor in and after low temperature (Schmid and Keller, 1980; Sichkar, 1988). As mentioned at the beginning, in Hokkaido, cold-weather damage in soybean production became more serious as the cultivated area expanded from the southern to the northern part of Hokkaido. Soybean production became unstable in the U.S as well for the same reason; as soybean production expanded to northern latitudes and higher altitudes, low temperature limited its productivity (Seddigh et al., 1988). Varietal differences to low night temperature were investigated (Seddigh et al., 1988). Relations of low night temperature with maturity, pod number, pod-filling speed and dry matter partitioning were reported (Seddigh and Jolliff, 1984a; Seddigh and Jolliff, 1984b).

The degree of damage by low temperature in soybeans depends on the growth stage. Onset of cooling treatment near the flowering stage has the largest effect on pod setting than other stages (Toriyama and Toyokawa, 1957; Saito and Takazawa, 1962). There is a strong negative correlation between the temperature in July, at the flowering stage of soybean, and grain yield (Horiguchi, 1980), and pod number (Yumoto and Tsuchiya, 1991) in Hokkaido. Many publications have documented that low temperature around the flowering stage decreases the total number of pods which resulted in lower grain yield of soybean. Cooling treatments

before (Toriyama and Toyokawa, 1957; Saito et al., 1970) or during the flowering period (Saito and Takazawa, 1962; Hume and Jackson, 1981; Michailov, V. G. et al., 1989a) cause severe flower damage and pod abscission. Goto and Yamamoto (1972) reported that exposure to low temperature for 15 days before flowering decreased pollen activity, consequently the density of pollen grains deposited on the stigma. A sensitivity of soybean flowers to low temperature is high when plenty of nitrogen fertilizers are applied (Hashimoto and Yamamoto, 1970a; Hashimoto and Yamamoto, 1970b). Shortage of photosynthate is a cause of flower abscission in normal condition due to floral competition (Hardman and Brun, 1971; Schou et al., 1978). However, low temperature decreases pod-setting rate in soybeans with only three flowers per plant adjusted by flower removal. Thus, photosynthate can not be a cause of flower abscission due to low temperature (Saito et al., 1970).

The severest cold-weather damage in Hokkaido in these fifty years was observed in 1993 mainly caused by flower abscission and pod-setting failure due to low temperature during the flowering stage. Yields in all of the varieties were remarkably decreased, but some breeding materials showed an excellent pod setting (Kurosaki et al., 1993), and Hayahikari was selected among the materials (Yumoto et al., 2000).

Varietal differences of the tolerance to low temperature during the flowering stage were investigated in Switzerland (Schmid and Keller, 1980), Poland (Szyrmer and Janicka, 1985) and Ukraine (Michailov et al., 1989a). Hume and Jackson (1981) and Szyrmer and Janicka (1985) reported that high pod-setting rate at low temperature is a trait of tolerant soybeans for the damage during low temperature at the flowering stage. Michailov et al. (1989b) reported that the number of seeds per plant is related to the cold-weather tolerance and the tolerance is controlled by one gene.

A temperature at the pod-filling stage in soybeans with earlier maturity is higher than that in ones with later maturity. In addition, a risk of early frost damage in varieties with earlier maturity is lower than that in ones with later maturity in cool regions. Varieties with earlier maturity have been developed in Hokkaido against insufficient grain filling due to low temperature in the pod filling stage. For example, with regard to yellow hilum variety, Toyosuzu was released in 1966 (Sunada et al., 1966). Thereafter Toyomusume (Sasaki et al., 1988), Toyokomachi (Sasaki et al., 1990) and Yukihome (Kurosaki et al., 2002; Tanaka et al., 2003) were released in 1985, 1988 and 2001, respectively. Every new variety matures about seven days earlier than the previous one. Moreover, yields of these varieties have been kept at the same level.

In high latitude countries, such as Poland (Szyrmer and Boros, 1996), Slovakia (Debre, 1996), Ukraine (Szyrmer and Boros, 1996) and the Far Eastern Russia (Zhiznevskaya et al., 1996), earlier varieties have been also developed to make use of a short growth period.

Many publications related to germination under low temperature documented world wide, although few researches of this area have been done in our country. Varietal differences and screening methods were reported in the U.S (Hesel et al., 1985; Unander et al., 1986), China (Li et al., 1989; Li et al., 1992), Canada (Littlejohn and Tanner, 1976) and Poland (Szyrmer and Szczepanska, 1982). Relations of germination under low temperature with seed shape, oil content and maturity groups were investigated. Soybean cultivars with small and kidney-shaped seeds, black seed coats, non-luster of seed coats, low oil percentage and high linoleic/oleic acid ratio generally showed higher tolerance (Li et al., 1989). There was no close relationship between maturity groups and a level of tolerance (Littlejohn and Tanner, 1976).

Cultural management such as a deep irrigation (Sakai, 1949; Satake et al., 1988) and an application of

proper amount of nitrogen fertilizer (Amano and Moriwaki, 1984) is effective against cold weather damage in rice plants. On the other hand, it was reported that an application of phosphorus is effective for poor growth due to low temperature in the early stage of growth because phosphorus promotes a growth of plant (Hashimoto and Yamamoto, 1974), but other cultural controls to reduce damage by low temperature are unknown in soybeans.

1.2 Occurrence of cold weather damage in Hokkaido.

Fig. 1-1 shows soybean yields in the Tokachi District from 1956 to 2004. Black dots indicate cold-weather damaged years. There were 12 times of the damaged years in 49 years. Thus, cold weather damage occurs at an average of once every four years.

Sasaki and Kamiya (1984) sorted out data of yield tests for approximately 40 years in the Tokachi Agricultural Experiment Station and degrees of cold-weather damage of the three types mentioned above can be calculated as follows.

1) Degree of damage due to poor growth $= 100 - 1/2(A + 100/B \times 100)$

A: plant height in the early stage of growth, B: days from planting to maturity.

2) Degree of damage due to flower abscission and pod setting failure

$= 100 - ((2 \times C) / (D + E) \times 100)$

C: number of pods in the maturity, D: plant height in the maturity, E: number of main stem nodes in the maturity.

3) Degree of damage due to insufficient grain filling $= 100 - F$

F: one hundred grain weight.

Each value is the percentage of the averaged years.

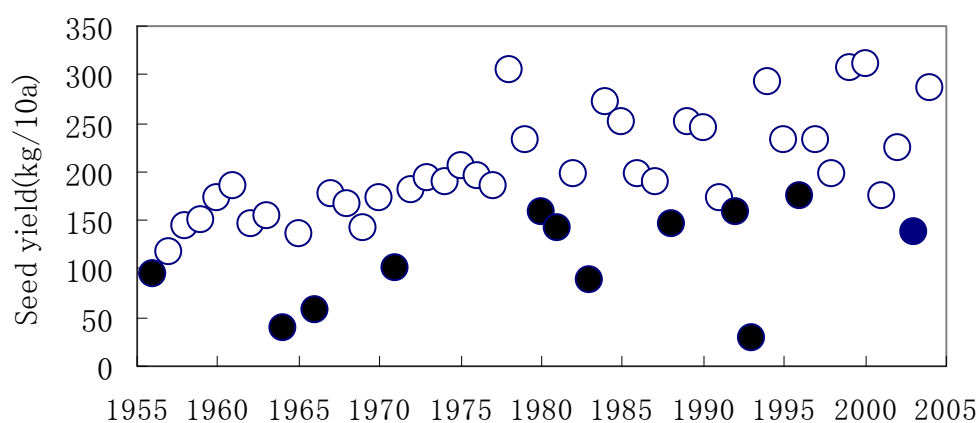


Fig.1-1. Average seed yield of soybean in Tokachi district. year
(Hokkaido Statistics and Information Office)
○: Non-cold-weather damaged year ●: Cold-weather damaged year

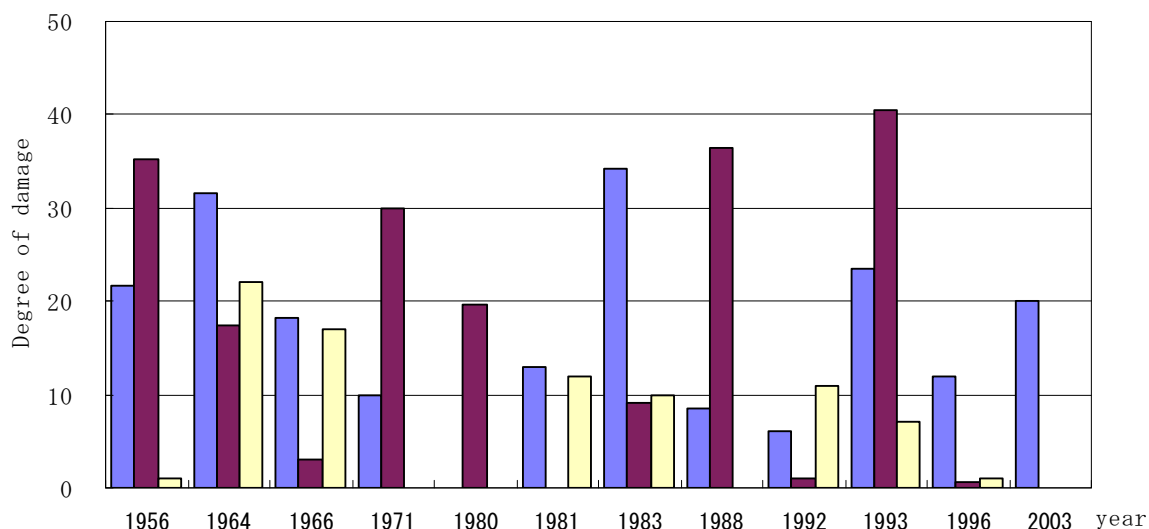


Fig.1-2. Degree of damage by three factors in cold-weather damaged years in Tokachi Agricultural Experiment Station.

Calculation of degree of cold-weather damage was followed by Sasaki and Kamiya (1984).

1) Degree of damage due to poor growth = $100 - 1/2(A + 100/B \times 100)$

A: plant height in the early stage of growth, B: days from planting to maturity.

2) Degree of damage due to flower abscission and pod setting failure = $100 - ((2 \times C)/(D+E) \times 100)$

C: number of pods in the maturity, D: plant height in the maturity, E: number of main stem nodes in the maturity.

3) Degree of damage due to insufficient grain filling = $100 - F$ F: one hundred grain weight.

Each value is percentage of the averaged years.

■ Poor growth ■ Flower abscission and pod setting failure ■ Insufficient grain filling

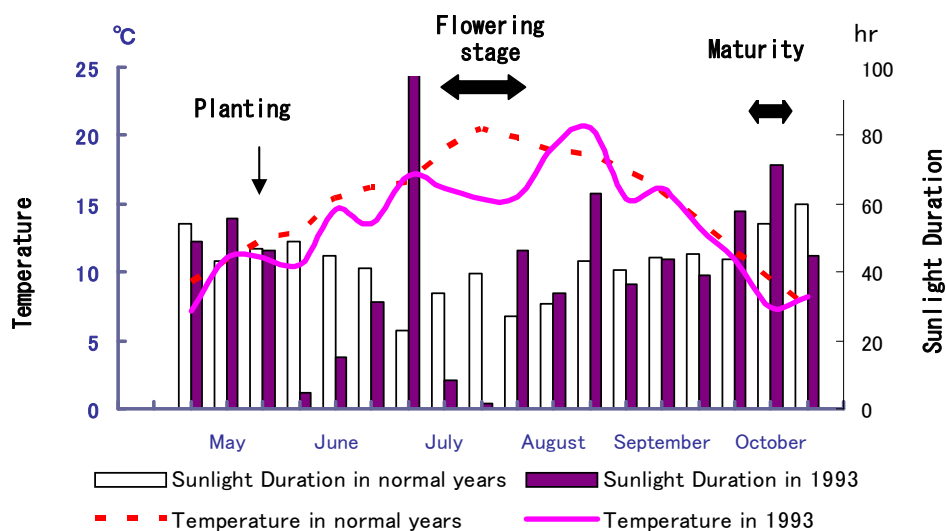


Fig. 1-3. Average daily temperature and sunlight duration every 10 days during soybean growth in Memuro (Tokachi District) in 1993 (severe cold-weather year).

Arrows indicate soybean growth stage.

Fig. 1-2 indicates the degree of damage caused by low temperature in 12 cold-weather damaged years according to the calculation by Sasaki and Kamiya (1984). In two of the 12 years with cold-weather damage, soybeans were damaged by one of the three factors mentioned above alone. In the other ten years of damage, the damage was caused by a combination of two or three of the factors. The frequency of the years with damage by poor growth was the highest, but the damage caused by flower abscission and pod-setting failure was very severe.

Mean temperature from pre-anthesis to flowering stage (mid of July to mid of August) transited 2 to 3 °C below the average in cold-weather damaged years mainly due to flower and pod abscission. And sunlight duration of the period was shorter. Especially, in 1993 (Fig.1-3), mean temperature of the flowering period was 15 °C, approximately 6 °C below the average, and sunlight duration in the end of July was only 1.4 hrs (4 % of the average), flower abscission and pod setting failure were remarkable. In addition, not only flower abscission but also poor growth and insufficient pod filling did the most severe damage to soybeans in these fifty years. Very severe flower abscission and pod setting failure were observed in all of the soybean varieties, except a few breeding materials. Thus, damage of seed yield due to flower abscission and pod setting failure can be enormous, therefore, to establish the countermeasure is important to stabilize soybean production in Hokkaido.

In this dissertation, factors controlling cold-weather damage during the flowering stage in soybeans and mechanism of cold-weather tolerance were examined to improve soybean production in view of breeding and genetics. Environmental factors controlling response of soybeans to cold weather damage by low temperature and less sunshine at the flowering stage was also investigated. The effect of low temperature on the pod setting during and after low temperature was compared between cold weather tolerant and the sensitive varieties.

Chapter 2 Effects of low temperature and shading during flowering on the yield components in soybeans.

The cold-weather damage in the Tokachi district was caused by both low temperature and insufficient sunlight. Numerous publications documented that the growth of soybean is sensitive to temperature and shading. Low temperature at the flowering stage influences pod setting (Saito et al., 1970; Duke et al., 1979; Hume and Jackson, 1981; Hayashi et al., 1998). Exposing soybeans to low temperature affects a wide range of physiological processes, including photosynthesis (Duke et al., 1979; Marowitch, et al., 1986; Purcell et al., 1987; Wang et al., 1997), carbohydrate metabolism (Thomas et al., 1981; Marowitch et al., 1986; Purcell et al., 1987), nitrogen fixation (Duke et al., 1979; Takahashi and Asanuma, 1996), and pollination (Goto and Yamamoto, 1972).

Shading reduces the number of pods (Mann and Jaworski, 1970; Tanaka et al., 1980; Ishikawa et al., 1984; Jiang and Egli, 1993) and the amount of nitrogen accumulation because of a reduction in nodule and root growth in the soybean plant (Tanaka et al., 1980). While, Allen (1975) reported that reducing exposure to sunlight by 30 % may be favorable during the excessively hot mid-day because it reduces soil and air temperature, wind speed, and water use. However, few attempts have been made on the effects of both low temperature and shading.

This experiment was conducted to determine the effects of low temperature, shading at normal temperature, and shading at low temperature using soybean cultivars with two different tolerance levels.

Materials and Methods

Plant materials

Two determinate cultivars, which have similar maturity, plant type and seed yield in normal years, were chosen for the experiment. Both varieties were developed at the Hokkaido Prefectural Tokachi Agricultural Experiment Station. One was Toyomusume with a yellow seed coat and hilum, gray pubescence and purple flowers (*I/I*, *t/t*, *r/r*, *O/O*, *W1/W1*; genotypes were summarized by Palmer and Kilen, 1987), which is a standard low-temperature sensitive variety. This variety was released in 1986 (Sasaki et al., 1988) and has been a main one grown in Hokkaido because of the excellent seed quality.

The other was Hayahikari, which has a yellow seed coat and brown hilum, tawny pubescence and white flowers (*i-i*/ *i-i*, *T/T*, *r/r*, *O/O*, *w1/w1*). Hayahikari showed the lowest seed yield reduction among soybean varieties and lines in 1993, where soybean was most severely damaged by cold weather in the past 50 years (Kurosaki et al., 1993). Field experiments in cooler locations and the experiments in a phytotron kept at low temperature also showed that Hayahikari is highly tolerant to cold weather. As a result, Hayahikari was released in 1998 as one of the most cold weather tolerant varieties (Yumoto et al., 2000).

Plant culture

Experiments were conducted from May to October in 1997 at the Hokkaido Prefectural Tokachi Agricultural Experiment Station (42° 55' N, 143° 03' E, 95 m above sea level). On 27 May, twelve seeds per pot were planted in pots (1/2000 a) filled with dry andosol soil supplemented with a synthetic fertilizer (0.6 N- 5.3 P₂O₅-2.7 K₂O kg a⁻¹). The plants were thinned to two per pot, two weeks after emergence.

The plants were grown in an experimental facility under a plastic roof without walls. The average temperature during soybean growth was 14.5 °C and that in the flowering season (20 July to 30 August) was 18.3 °C (Fig. 2-1), 0.7 °C and 1.2 °C lower than the average of the last ten years, respectively. The sunlight duration of the flowering season was 140.7 hours, 5.8 hours less than the average. During the half of the flowering season, the weather was clear. The growing conditions were those of a normal year, not a cold year.

Cooling and shading treatments

Forty plants of each variety were grown in 20 pots in an experimental facility. Ten plants each of the two varieties were exposed to low temperature in a phytotron (cooling treatment), shaded at a normal temperature (shading treatment) or shaded at low temperature (shading + cooling treatment) during the four-week flowering season. In the years with cold-weather damage, the average and the minimum temperature every 10 days during the flowering season were from 16 to 19°C and from 12 to 14°C, respectively. Therefore, the temperature in the phytotron for cooling treatment was set at 18°C day / 13°C night. The shading treatment was done by stretching screens horizontally near the roof of the phytotron or the experimental facility. The shading level was about 50%. Each treatment started on 21 July in Toyomusume and on 22 July in Hayahikari. The remaining 10 plants of each variety were used as the control.

At maturity, the number of main stem nodes, the number of branches, the total number of nodes, the numbers of pods, ovules and grains per pod, and the weight of seeds were measured for each plant.

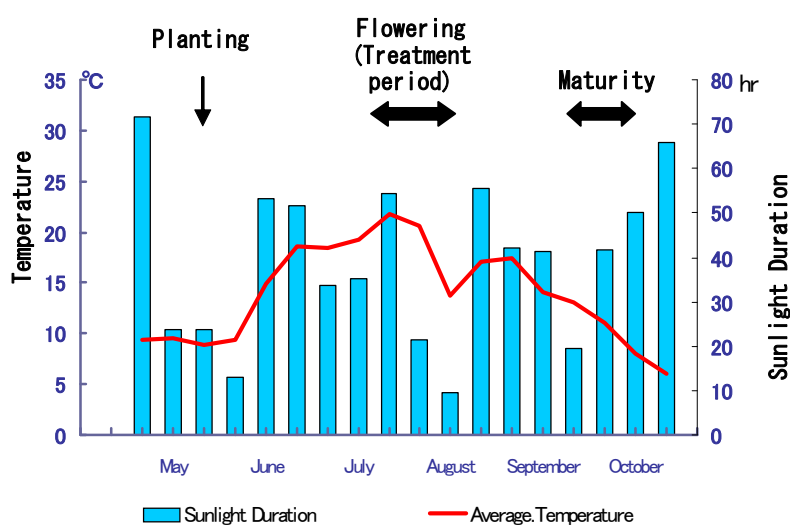


Fig. 2-1. Average daily temperature and sunlight duration every 10 days during soybean growth in Memuro in 1997. Arrows indicate soybean growth stage.

Leaf temperature measurement

Plant temperature has a relation with the rates of photosynthesis and translocation in soybeans (Marowitch et al., 1986). To measure the effect of shading with low temperature, the temperature of the leaf at the top of Hayahikari plants was recorded in the control, the cooling treatment and the shading + cooling treatment. The data were recorded for four days both on clear and cloudy days between 13:00-14:00 by a spot thermometer (MINOLTA: Type 505).

Plant arrangement and data analysis

Pots were randomized in both phytotron and experimental facility and repositioned at least once a week. The data of the number of main stem nodes, the number of branches, the total number of nodes, the numbers of pods, ovules and grains per pod, the fertilization rate, 100 seed weight (seed size) and the seed yield were subjected to the t-test.

Results

1. Effects of cooling and shading treatments

In the control conditions, Toyomusume matured on 5 October, and Hayahikari 4 days earlier on 1 October. The shading treatment did not greatly affect the maturity date in either variety; it delayed maturity by 3 days in Toyomusume and hastened by 2 days in Hayahikari. The cooling treatment and the shading + cooling treatment delayed the maturity date by almost 20 days in both varieties. Effects of cooling and shading treatments on the vegetative factors were not large in either variety. Neither cooling nor shading treatment significantly affected the number of main-stem nodes per plant, the number of branches per plant and the total number of nodes per plant in either variety (Fig. 2-2, Table 2-1).

Although the seed yield was approximately the same, about 20 g per plant, in both varieties under the control conditions, it was higher in Hayahikari than in Toyomusume after the cooling treatment. The seed yield after the shading treatment at normal temperature was nearly the same in the two varieties (Fig.2-3).

Table 2-1. Effects of cooling and shading treatments: t-test results.

Variety	Main stem nodes per plant	Branches per plant	Total nodes per plant	Pods per plant	Ovule s per pod	Grains per pod	Fertilization rate	100 seed weight	Seed yield per plant
Toyomusume									
Control vs Cooling	ns	ns	ns	**	**	**	**	**	**
Control vs Shading	ns	ns	ns	*	ns	ns	ns	ns	*
Cooling vs Shading+ Cooling	ns	ns	ns	*	ns	ns	ns	**	ns
Hayahikari									
Control vs Cooling	ns	ns	ns	ns	ns	**	**	ns	**
Control vs Shading	ns	ns	ns	**	**	ns	**	ns	*
Cooling vs Shading+ Cooling	ns	ns	ns	*	ns	ns	ns	ns	*

*, ** and ns indicate significant at $P = 0.05$, $P = 0.01$ and not significant difference, respectively.

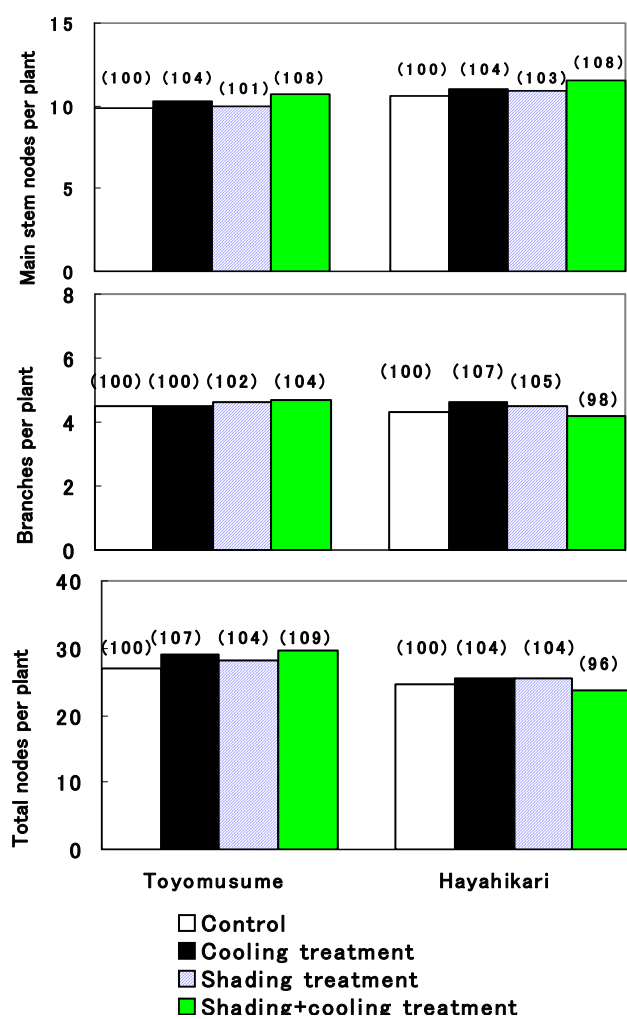


Fig. 2-2. Effects of cooling and shading treatments on vegetative organs of soybeans. Numbers in parentheses are percentage of the control.

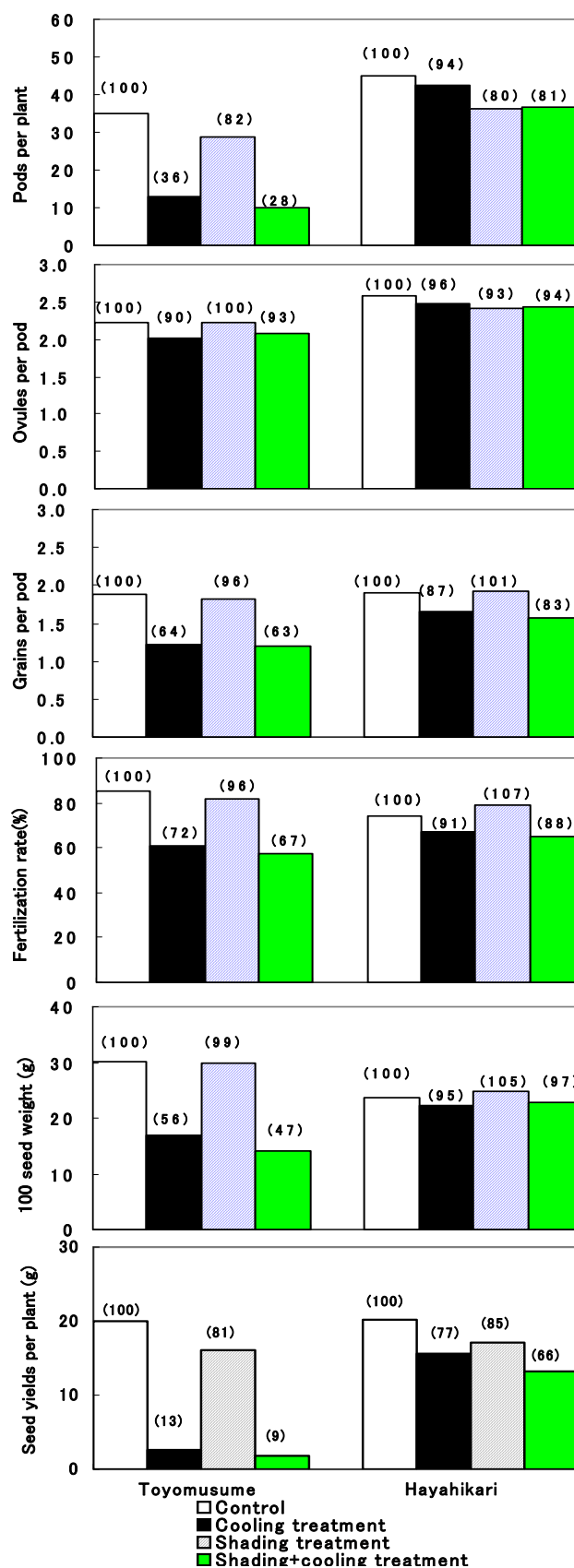


Fig.2-3. Effects of cooling and shading treatments on yield components of soybeans. Fertilization rate = (Grains per pod) / (Ovules per pod) × 100. Numbers in parentheses are percentage of the control.

The seed yield in Toyomusume was reduced to 2.6 g (13 % of the control) by the cooling treatment, but that in Hayahikari only to 15.6 g (77 % of the control). Thus, the results confirmed that Hayahikari is more tolerant to low temperature than Toyomusume. In Toyomusume, all yield components, the number of pods per plant, the numbers of ovules and grains per pod, the fertilization rate and 100 seed weight, were reduced significantly ($P < 0.01$) by the cooling treatment. In Hayahikari, however, only the number of grains per pod and the fertilization rate were reduced significantly ($P < 0.01$). The largest difference between the two varieties in the response to the cooling treatment was the number of pods per plant, which was reduced from 35.2 to 12.8 (36 % of the control) in Toyomusume, but only from 45.2 to 42.5 (94 % of the control) in Hayahikari. A large difference was also observed in 100 seed weight. It was reduced by the cooling treatment from 30.2 to 16.8 g (56 % of the control) in Toyomusume, and from 23.6 to 22.3 g (95 % of the control) in Hayahikari. The number of ovules, the number of grains per pod and the fertilization rate in Toyomusume were reduced by the cooling treatment to 90, 64 and 72 % of the control, respectively, while those in Hayahikari were reduced only to 96, 87 and 91 % of the control, respectively.

The number of pods per plant was significantly reduced by the shading treatment (to about 80 % of the control) in both varieties ($P < 0.05$ in Toyomusume and $P < 0.01$ in Hayahikari), which resulted in a significantly ($P < 0.05$) lower seed yield. The number of ovules per pod was significantly ($P < 0.01$) decreased by the shading treatment in Hayahikari but not in Toyomusume. The number of grains per pod was unaffected by the shading treatment in both varieties. No effect of shading was found in 100 seed weight in either variety. As a result, the rates of reduction of seed yield and that of the number of pods per plant by the shading treatment were approximately the same in both varieties (81 and 82 % in Toyomusume; 85 and 80 % in Hayahikari).

The reductions in the numbers of ovules and grains per pod and the fertilization rate by the cooling treatment were not significantly influenced by shading during the cooling treatment (shading + cooling treatment). However, the number of pods per plant and seed yield after the cooling treatment were significantly ($P < 0.05$) reduced by shading during the cooling treatment. The number of pods per plant was reduced from 12.8 (36 % of the control) to 10.0 (28 % of the control) in Toyomusume, and from 42.5 (94 % of the control) to 36.6 (81 % of the control) in Hayahikari. The seed yield was decreased from 2.6 g (13 % of the control) to 1.8 g (9 % of the control) in Toyomusume, and from 15.6 g (77 % of the control) to 13.2 g (66 % of the control) in Hayahikari.

2. Leaf temperature

In the control, the leaf temperature of Hayahikari was the same as the outside temperature during cloudy weather (Table 2-2), but it was markedly higher (29.5 °C) than the outside temperature (24.5 °C) during clear weather.

In the phytotron, during cloudy weather, the leaf temperature was almost the same as the controlled air temperature (18 °C) irrespective of shading. However, during clear weather, although the leaf temperature of the cooling treatment was about 6 °C higher (24.6 °C) than the air temperature, that of the shading + cooling treatment (18.6 °C) was nearly the same as the air temperature. Thus, the leaf temperature during the cooling treatment was markedly raised by sunlight but not under shading.

Table 2-2. Leaf temperature of Hayahikari under clear and cloudy weather.

Outside Temperature (°C)		Amount of sunlight (kcal h ⁻¹ m ⁻²)		Leaf Temperature (°C)					
Clear	Cloudy	Clear	Cloudy	Control		Shading + Cooling treatment		Cooling treatment	
				Clear	Cloudy	Clear	Cloudy	Clear	Cloudy
24.5±0.9	21.3±0.3	1062±24.6	350±172.6	29.5±1.7	21.8±0.5	18.6±0.2	18.0±0.0	24.6±0.9	18.3±0.8

Data are means ± standard errors.

Discussion

The reproductive growth was greatly affected by low temperature, but the vegetative growth was not in either variety. The number of main stem nodes per plant, the number of branches per plant and the total number of nodes per plant after the cooling treatment were approximately the same those in the control conditions in both varieties. It is assumed that the differentiation of vegetative growth is almost completed at the beginning of the flowering period, and low temperature increases the partitioning of total biomass to vegetative organs, while it decreases its partitioning to the reproductive organs (Seddigh and Jolliff, 1984a; Wang et al., 1997). Thus, the effect of low temperature during the flowering period on vegetative organs is small.

Although low temperature significantly damaged seed yields in both varieties, Hayahikari showed higher tolerance than Toyomusume. This varietal difference was due to different reduction rates in the number of pods per plant, the number of grains per pod, the fertilization rate and 100 seed weight. This means that the difference between Hayahikari and Toyomusume in the decrease of seed yield due to cold weather is derived from the tolerance to low temperature of several components, not just one component, related to yield in Hayahikari.

The most drastic difference between Toyomusume and Hayahikari in the response to the cooling treatment was the reduction in the number of pods per plant. It was significantly reduced in Toyomusume, but not in Hayahikari. This indicates that pod number is the most important component responsible for cold weather tolerance. It can be considered that Hayahikari has a higher pod-setting ability in cold weather than Toyomusume. However, this result does not mean that Hayahikari has a superior pod-setting ability during low temperature period. In other words, there is a possibility that most of Hayahikari's pods were set after the low-temperature period. Further investigation about how Hayahikari's pods are set in cold weather is necessary.

The other reproductive growth components, the numbers of ovules and grains per pod and the fertilization rate, also varied with the cultivars. The number of grains per pod and the fertilization rate were significantly reduced by the cooling treatment in both varieties, although the degree of the reduction in Toyomusume was greater than that in Hayahikari. More generally, the results showed that low temperature affects the process of fertilization: the number of unfertilized ovules was increased, therefore the number of grains per pod and the fertilization rate were decreased by the cooling treatment. This finding is also supported by the study of Goto and Yamamoto (1972), who found that cooling treatment before flowering decreased pollen activity and pollen density per stigma, and thus increased the number of unfertilized flowers.

Low temperature decreased the seed size more in Toyomusume than in Hayahikari. The 100 seed weight of Toyomusume in the control was 30.2 g, which was about 7 g heavier than that of Hayahikari. However, the 100 seed weight of Toyomusume was about 6 g lighter than that of Hayahikari after the cooling treatment, showing

a drastic reduction of seed weight by low temperature. In this experiment, the pod size and seed size were approximately equal in the two varieties at the end of the cooling treatment (data not shown), and the period from the end of the cooling treatment to maturity was the same in the two varieties. Therefore, it is assumed that the difference in the reduction of the seed weight by the cooling treatment in the two varieties was caused by the difference in pod filling after the cooling treatment.

Note that the number of pods per plant was reduced by the shading treatment similarly in the two varieties. In both Toyomusume and Hayahikari, it was decreased by about 20 % compared with the control. Mann and Jaworski (1970) and Jiang and Egli (1993) also reported that shading decreased the number of pods per plant. It is supposed that insufficient sunlight decreases photosynthetic activity and reduces assimilates, which results in pod abscission as Schou et al. (1978), Egli and Zhen-wen (1991) described. Both varieties demonstrated a parallel reduction of the number of pods per plant by shading, and no major difference was found in the other yield components. Therefore, the seed yield reduction was almost parallel to the reduction of the number of pods per plant in both varieties. This result shows that there is no difference between the two cultivars in the susceptibility to shading at normal temperature.

Hayahikari showed a high tolerance to the cooling treatment, but less tolerance to the shading + cooling treatment. Furthermore, the seed yield after the shading + cooling treatment was lower than that after the cooling treatment in both Toyomusume and Hayahikari. The damage caused by the shading + cooling treatment appeared to be a combination of that caused by shading and cooling. In both varieties, the reduction of the numbers of ovules and grains per pod, and the fertilization rate by the shading + cooling treatment paralleled the reduction by the cooling treatment. However, pod number was decreased more strongly by the shading + cooling treatment than the cooling treatment. Shading at a normal temperature significantly decreased the number of pods per plant but did not seriously affect other yield components in either variety. A greater reduction in the number of pods per plant by the shading + cooling treatment than by the cooling treatment shows the combined effects of cooling and shading.

This phenomenon can be partially explained by leaf temperature. According to Marowitch et al. (1986), the lower the plant temperature, the lower the rate of photosynthesis and translocation of assimilates in soybeans during cold weather. In cloudy weather, the leaf temperature during the cooling treatment was unaffected by shading. In clear weather, however, the leaf temperature during the cooling treatment (24.6°C) was lowered to 18.6°C by shading. In other words, the leaf temperature during the cooling treatment was 6°C higher than that during the shading + cooling treatment in clear weather. Therefore, the cause of the severe decrease in the number of pods and seed yield by the shading + cooling treatment can be considered as follows. When the low temperature is combined with sufficient sunlight during the flowering period, which is an essential stage for pod setting, the leaf temperature may be high enough for photosynthesis and translocation of assimilates. However, when the low temperature is combined with insufficient sunlight, the temperature may be too low to support photosynthesis and translocation of assimilates, cause severe damage to plants.

In conclusion, this chapter showed that low temperature during the flowering season markedly reduced seed yield and shading during the same period reduces pod number. Shading combined with cooling treatment causes greater damage than either low temperature or shading treatment alone. A varietal difference in the seed yield after the cooling treatment is attributed to the difference in the response of yield components to the low temperature, especially the number of pods per plant.

Chapter 3 Differences in pod setting pattern during and after low temperature between the cold-weather sensitive and the tolerant varieties.

Several researchers documented a varietal difference in the response to low temperature during the flowering stage in field experiments. Szyrmer and Janicka (1985) counted the total number of flowers and pods in cold-weather years, and concluded that cold-weather tolerant varieties show higher pod-setting rates. In the previous chapter, it was shown that the yield components – number of pods per plant, ovules per pod, grains per pod and seed size – of a cold-weather sensitive variety are more susceptible to cold-weather damage than those of a cold-weather tolerant variety. And it was also clarified that the greatest factor giving tolerance to low temperature is pod number.

As pod number is the key factor of cold-weather tolerance, it is important to study the pod setting during and after low temperature. In this chapter, the effects of short-term and long-term exposure to low temperature on pod-setting rate in the cold-weather sensitive and the tolerant varieties were examined.

Materials and Methods

A cold-weather sensitive standard variety “Toyomusume” and a cold-weather tolerant standard variety “Hayahikari” were used. Characteristics of the two varieties are given in Chapter 2. Experiments were conducted from May to October in 1996 at the Hokkaido Prefectural Tokachi Agricultural Experiment Station. On 23 May, twelve seeds were planted in pots (1/2000 a), filled with dry Andosol supplemented with a synthetic fertilizer (0.6 N- 5.3 P₂O₅-2.7 K₂O kg a⁻¹) and watered regularly. Two weeks after emergence, pots were thinned to two plants per pot.

During experiments, pots were grown in an experimental facility having a plastic roof without walls. The average temperature during the growth period was 15.1 °C, which was 1.0 °C lower than the average temperature of the last ten years.

Thirty plants of each variety were grown in pots (two plants per pot) in the experimental facility. For cooling treatments, 20 plants were transferred to a phytotron. The air temperature in the phytotron was set at 18 °C/13 °C(day/night), the same as in Chapter 2. The cooling treatments started at the beginning of flowering, 19 July in Toyomusume and 22 July in Hayahikari. As a result of Chapter 2, shading during cooling treatment stimulates pod abscission, the plants were shaded (50 % shade) during the cooling treatments. The cooling treatment regimes in the phytotron are shown in Table 3-1. For the short-term treatment group, the low temperature stress was applied for two weeks. For the long-term treatment group, it was applied for four weeks. Ten plants of each variety were assigned to each group. The remaining ten plants of each variety were used as the control.

From the beginning of flowering for six weeks during which all flowers opened, the number of flowers in four out of the ten plants in each group were counted and marked with different colored strings. The total number of flowers was calculated for each week. Flowers were labeled within a week after opening. Flowers that opened during the 1st, 2nd, 3rd, 4th, 5th and 6th week after the initial flower opened are referred to as F1W, F2W, F3W, F4W, F5W and F6W, respectively. At maturity, the number of pods and weight of seeds were measured for each plant.

Pots in the phytotron and in the experimental facility were randomly rearranged at least once a week. The significance of the difference between the treatments in each variety was determined by the Tukey-Kramer multiple method for the number of pods and weight of seeds. The pod-setting rate each week was calculated as follows:

$$\text{The pod-setting rate (\%)} = (\text{The number of pods}) / (\text{The number of flowers}) \times 100$$

Table 3-1. The cooling treatment regimes.

Treatment	Week 1 to Week 2	Week 3 to Week 4
Control	23/18°C*	23/18°C
Short-term	18/13°C + Shading**	23/18°C
Long-term	18/13°C + Shading	18/13°C + Shading

Treatment was started at the beginning of flowering.

*: Air temperature (day/night) **: 50% shaded.

Results

1. Effect of low temperature on the number of pods per plant and seed yield.

Although the seed yield in the control group was nearly the same in both varieties, Hayahikari showed higher tolerance to the low temperature than Toyomusume (Table 3-2). The cooling treatments significantly ($P < 0.05$) decreased the seed yield in both varieties. However, there was a clear varietal difference. In the short-term treatment, the seed yield in Toyomusume was 81 % of the control, but that in Hayahikari was 87 %. A greater varietal difference was observed in the long-term treatment, in which seed yield was 25 % in Toyomusume, but only 69 % in Hayahikari.

The number of pods per plant was not significantly decreased by the short-term cooling treatment in both varieties, but was significantly reduced by the long-term treatment. Toyomusume was affected more than Hayahikari. In Toyomusume, the number of pods per plant was 99 % of the control in the short-term treatment and 55 % in the long-term treatment, while they were 108 % and 93 %, respectively in Hayahikari.

2. Effect of low temperature on flowering and pod setting.

The total number of flowers per plant in the control was 77.3 and 76.4 per plant in Toyomusume, in Hayahikari, respectively, and the cooling treatments increased the number in both varieties (Fig. 3-1). The total number of flowers was 86.3 per plant in the short-term treatment, and 92.4 in the long-term treatment in Toyomusume, and 84.8 and 92.6, respectively in Hayahikari.

Fig. 3-2 shows the effect of low temperature on flowering and pod setting at each week. In the control group, F1W and F2W accounted for a major proportion of the total flowers in both varieties, and flowering was completed within four weeks. Cooling treatments prolonged the period of flowering to six weeks (except for the short-term treatment in Hayahikari), but no plants opened flowers after the seventh week. In the short-term treatment of both varieties, fewer flowers opened in F1W and F2W than in the control, but the number of flowers increased in the third and fourth weeks in both varieties.

In the control, pod setting completed within two weeks in both varieties, and no pod was set in the third

and fourth weeks. The pod-setting rates of F1W and F2W in the control were 60 % and 41 %, respectively in Toyomusume, and 72 % and 36 %, respectively in Hayahikari (Fig. 3-3). In the short-term treatment, pods set even in the fourth and fifth weeks, and many pods were set after the cooling treatment in both varieties. Similar to the number of flowers, the cooling treatment decreased the number of pods during the short-term treatment (F1W and F2W) compared to the control in both varieties. For F1W, however, the effect of the low temperature on pod setting was different with the variety. The pod-setting rate of F1W was 33 % in Toyomusume and 83 % in Hayahikari. The effect of short-term treatment on the pod-setting rate of F2W was almost the same in both varieties. Little damage was observed in F2W, and a similar number of pods developed in both varieties. The pod-setting rate was 45 % in Toyomusume, and 51 % in Hayahikari. The pod-setting rate of F3W in the short-term treatment was as high as that of F2W in the control in both varieties, i.e., 56 % in Toyomusume and 52 % in Hayahikari (Fig. 3-3).

In the long-term treatment, the number of flowers in F3W and F4W were higher than those in the control (Fig. 3-2). Many flowers opened in the fifth week after the cooling treatment in both varieties. In the long-term treatment, the number of F3W was less than that in the short-term treatment. However, the number of F4W was similar in both long- and short-term treatments. In the fifth week, the number of flowers in the long-term treatment was much higher than that in the short-term treatment (Fig 3-2).

The effect of the long-term treatment on pod setting was also greater than that of short-term treatment. In the first two weeks, the effect of the low temperature on pod setting varied with the variety; the pod-setting rates of F1W and F2W were very low in Toyomusume, but those in Hayahikari were approximately equal to those in the control (Fig. 3-3). The pod-setting rate in the long-term treatment was 9 % in F1W and 8 % in F2W in Toyomusume, while they were 60 % and 59 %, respectively in Hayahikari.

In F3W, the pod-setting rate was 52 % in Hayahikari, but it was only 26 % in Toyomusume (Fig. 3-3). In F4W, however, the number of pods and the pod-setting rate in the long-term treatment were approximately the same (about 30 %) in both varieties.

Fewer pods were set after the cooling treatment in the long-term treatment than the short-term treatment for both varieties. Hayahikari set pods 93 % of those in control by the fourth week, and little pod setting was observed in the fifth or sixth weeks. In contrast, the pod number of Toyomusume by the fourth week was only 45 % of that in the control. Toyomusume continued to set some pods in the fifth and sixth weeks. However, the pod-setting rates in Toyomusume were 23 % in F5W and 12 % in F6W. Thus, the total number of pods in Toyomusume in the long-term treatment was only 55 % of that in the control at maturity.

Table 3-2. Effects of cooling treatments on pod number and seed yield.

Variety	Treatment	First flower opened Date	Last flower opened Date	Maturity Date	Pod number per plant	Seed yield per plant (g)	
Toyomusume	Control	19,July	14,Aug	1,Oct	29.5 (100)	a	17.4 (100) a
	Short-term	19,July	24,Aug	5,Oct	29.2 (99)	a	14.1 (81) b
	Long-term	19,July	28,Aug	15,Oct	16.1 (55)	b	4.3 (25) c
Hayahikari	Control	22,July	16,Aug	25,Sept	35.1 (100)	ab	17.3 (100) a
	Short-term	22,July	21,Aug	30,Sept	37.9 (108)	a	15.0 (87) b
	Long-term	22,July	30,Aug	14,Oct	32.8 (93)	b	11.9 (69) c

See Table 3-1 for an account of cooling treatments.

Within columns, means followed by the same letter are not significantly ($p=0.05$) different to the Tukey-Kramer multiple method.

Parentheses are percent of control.

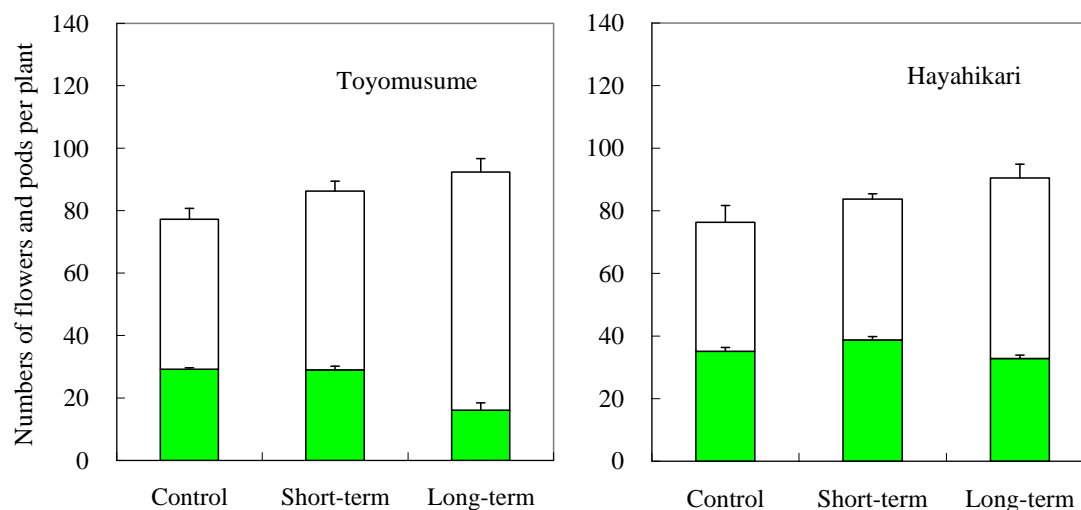


Fig 3-1. Total numbers of flowers and pods per plant in the plants exposed to low temperatures for two weeks (short-term treatment) and four weeks (long-term treatment).

Pods

Flowers

Vertical bars: standard error

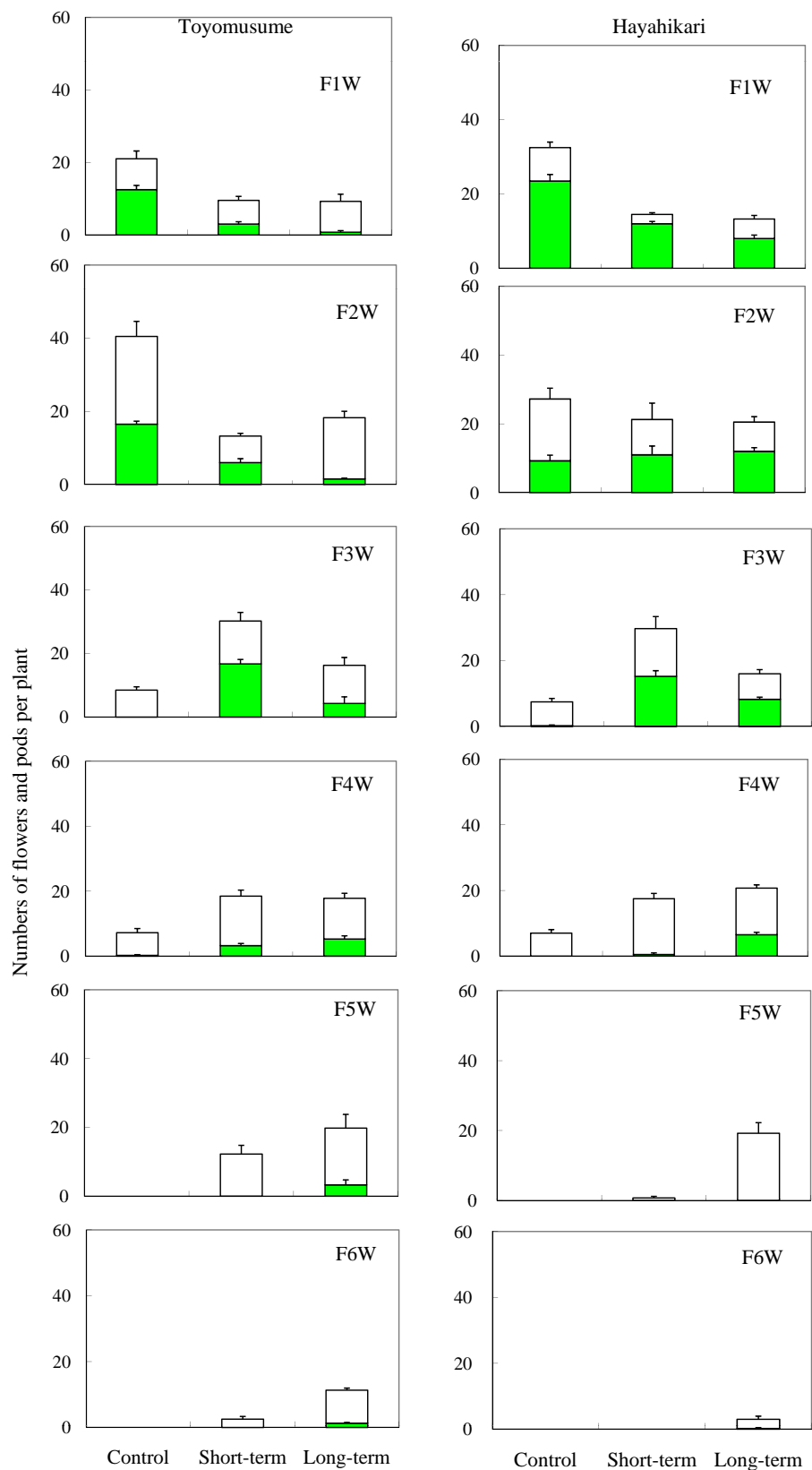


Fig. 3-2. Numbers of flowers opened and pods set per plant in each week in the control, short-term and long-term treatments. F1W, F2W, F3W, F4W, F5W and F6W are flowers that opened during the 1st, 2nd, 3rd, 4th, 5th, and 6th week after the start of flowering, respectively .

Pods

Flowers

Vertical bars: standard error

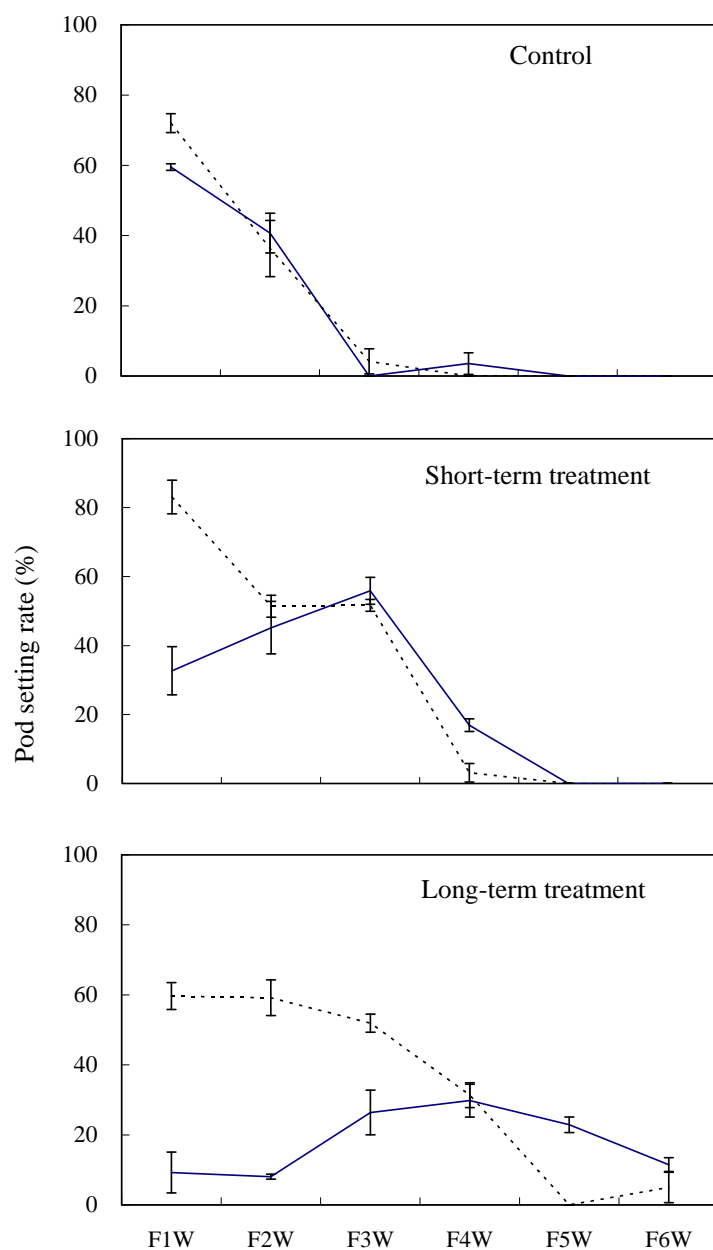


Fig. 3-3. Pod setting rate of F1W~F6W in the control, short-term and long-term treatments. F1W, F2W, F3W, F4W, F5W and F6W are flowers that opened during the 1st, 2nd, 3rd, 4th, 5th, and 6th week after the start of flowering, respectively .

Pod setting rate (%) = (number of pods) / (number of flowers) × 100

—— Toyomusume Hayahikari Vertical bars: standard error

Discussion

1. Effect of low temperature on pod number.

In this research, the number of pods per plant was not significantly decreased by the short-term cooling treatment in either variety. Saito (1972) reported similar results. He found that the total pod number was slightly reduced by two weeks of cooling treatment. In this study, however, the pod-setting rates of Hayahikari and Toyomusume were greatly different, even in the short-term treatment. The pod-setting rate of F1W in Hayahikari was 50 % higher than that in Toyomusume. The high tolerance of Hayahikari to low temperature could be attributed to the superior pod-setting ability under low temperature conditions. The difference in cold-weather tolerance between Hayahikari and Toyomusume was more evident in the long-term treatment, in which the pod number was 93 % of the control in Hayahikari, but only 55 % of the control in Toyomusume.

2. Effect of short-term cooling treatment on pod setting.

The total number of pods was not significantly decreased by the short-term treatment in either variety. The effect of the low temperature on pod setting was nearly the same in F2W in both varieties. However, the pod-setting rate of F1W in Hayahikari was as high as that in the control, although it was apparently lower in Toyomusume. As F1W were exposed to cooling treatment for two weeks, the pod-setting rate varies according to tolerance level even in the case of short-term low temperature exposure.

As described above, the total number of pods was not decreased by the short-term cooling treatment in either variety, because many pods were set after the treatment to compensate for the decreased pod number due to the cooling treatment. Approximately 70 % of the total pods in Toyomusume were set after the short-term treatment and 40 % in Hayahikari. This phenomenon can be explained by an increased number of flowers after the short-term treatment. Low temperature damaged flowers which opened during the cooling treatment in Toyomusume but not in Hayahikari. Suppressed flower development resulted in fewer flowers to bloom during the short-term cooling treatment in both varieties. After the short-term cooling treatment, the plants produced more flowers to compensate for decreased pod setting. As a result, the total number of flowers in the short-term treatment was higher than the control. After the cooling treatment, many flowers were able to set pods because they did not receive severe damage from the short-term exposure to low temperature. These results indicate that a lower rate of pod setting during short-term treatment can be compensated by pod setting after the cooling treatment.

3. Effect of long-term cooling treatment on pod setting.

A greater varietal difference in the number of pods per plant was observed in the long-term treatment than in the short-term treatment. The total pod number in Toyomusume was reduced nearly 50 % by the long-term cooling treatment, but that in Hayahikari was reduced only less than 10 %. The varietal difference in the total pod number in the long-term treatment was directly related to the difference in pod setting ability at low temperature. In Toyomusume, the pod-setting rates of F1W and F2W in the long-term treatment were less than one third of those in the short-term treatment. However, in Hayahikari, the pod-setting rates of F1W and F2W in the long-term treatment were only slightly less than those in the short-term treatment. Although the pod-setting rate of F4W in the long-term treatment was almost the same in both varieties, the pod-setting rate of F3W in the long-term treatment was about 20 % lower than that in the short-term treatment in Toyomusume. On the other hand, in Hayahikari, the pod-setting rate of F3W in the long-term treatment was almost equal to that in the short-term treatment. Thus, Hayahikari had a high pod setting ability, even in the

long-term low temperature. In the meantime, the long-term cooling treatment greatly reduced the pod setting ability of Toyomusume.

Toyomusume had poor compensative pod setting after the long-term cooling treatment. In cold-weather sensitive varieties, damage caused by low temperature for a short period can be compensated by pod setting after the stress. However, damage caused by low temperature over a long period cannot be effectively compensated. Flowers exposed to low temperature for a long-term before flowering seem to receive lethal damage and cannot develop into pods even after the removal of stress.

The varietal difference in the total pod number in the long-term treatment was attributed to the difference in the pod setting abilities of F1W and F2W. F1W and F2W in the long-term treatment were exposed to low temperature two weeks longer than those in the short-term treatment. The results show that low temperature for more than two weeks after flowering causes severe flower abscission in cold-weather sensitive varieties such as Toyomusume. On the other hand, Hayahikari showed a high tolerance to low temperature stress in pod setting.

In conclusion of this chapter, the total number of pods is not significantly decreased with a short-term low temperature exposure even in cold-weather sensitive varieties because of post-stress pod setting. However, the pod-setting ability during low temperature varied with the variety. Cold-weather tolerant varieties had a superior pod setting ability, but cold-weather sensitive varieties had a low pod-setting ability under low temperature conditions. In the case of long-term low temperature exposure, which significantly decreases the total number of pods for cold weather sensitive varieties, cold weather sensitive varieties often sustain damage not only during low temperature stress but also after it. On the other hand, cold weather tolerant varieties show a greater capacity for pod setting during low temperature stress.

Chapter 4 The mechanism of cold-weather tolerance during the flowering stage.

Abscission rates under normal conditions in soybean are much higher than those in other crops (Van Schaik and Probst, 1958). It was reported that causes of flower abscission are drought, fertilization failure and shortage of assimilates (Kato and Sakaguchi, 1954; Kato, 1964). However, flowers in plants in which the buds were removed to only three ones were abscised by low temperature during flowering stage, a shortage of assimilate is not a cause of flower abscission due to low temperature (Saito et al., 1970). Many publications have documented that low temperature during the flowering stage causes abnormal pod setting. Low temperature induce cleistogamy (Erickson, 1975; Thomas et al., 1981; Takahashi et al., 2001), which results in the development of many seedless pods at the top of the plant, and the presence of multi-carpelate and deformed pods (Hume and Jackson, 1981; Thomas et al., 1981). Development of pollen and pollination was affected by low temperature at the flowering stage (Goto and Yamamoto, 1972). However, few attempts have been made on analyzing the mechanism of cold-weather tolerance. In this chapter, pollen activity, pollination and fertilization were investigated to determine the mechanism of the tolerance using soybean cultivars with two different tolerance levels.

Materials and Methods

Experiment 4-1: Effect of low temperature on pollen germination ability.

A cold-weather sensitive variety, Toyomusume was cultured in 1996 as in Chapter 3. Twelve seeds were planted in pots (1/2000 a), filled with dry Andosol supplemented with a synthetic fertilizer (0.6 N- 5.3 P₂O₅-2.7 K₂O kg a⁻¹) and watered regularly. Two weeks after emergence, pots were thinned to four plants per pot.

The cooling treatment [15 °C/10 °C(day/night)] was applied for 11 days from the beginning of flowering. One flower per plant was sampled from eight plants every two days from the beginning of flowering. Three or four anthers per sampled flower were taken and left in artificial medium with 20 % sucrose and 1 % agar. The medium was left for 6 hours in a incubator kept at 25 °C, and germination rates were counted using a microscope with magnification $\times 20$. The rate of pollen germination was calculated as follows;

The pollen germination rate (%) = (the number of germinated pollen grains) / (the total number of pollen grains) $\times 100$.

Experiment 4-2: Effect of low temperature on pollination.

Toyomusume and a cold-weather tolerant variety, Hayahikari were cultured in 1996 as in Chapter 3. The cooling treatment [18 °C/13 °C(day/night)] was similarly applied for a week from the beginning of flowering. And anther length was measured in the day when first flower opened just before the cooling treatment.

Flowers were sampled every two days from the beginning of flowering for seven days. Stigmas were cut from the sampled flowers and stained with Cotton-blue solution. Then, the number of stained pollen grains on each stigma was counted using a microscope with magnification $\times 200$.

The flowers were classified according to the method used for Adzuki bean reported by Shimada (1990). Limited research has been done on the number of pollen needed for a successful fertilization in soybeans even

under optimal conditions, probably due to the miniscule size of the soybean stigma. Shimada categorized flowers of Adzuki bean by the number of pollen grains on stigma, which has several times larger stigma than soybean stigma, and reported that more than 100 pollen grains are required for a successful fertilization.

In this experiment, the flowers of soybean were classified into three categories: flowers having more than 100, those with 50 - 100 and those with less than 50 pollen grains on each stigma. A ratio of the flowers classified into the three categories was calculated. Since an error was assumed to follow a poisson distribution in this analysis, a logarithmic transformation was used to improve the homoscedasticity. The effect of the cooling treatment was tested by the likelihood ratio test. The procedure GENMOD of SAS was used for the calculation.

Experiment 4-3: Effect of low temperature on fertilization.

The materials and methods were the same as in experiment 4-2. The plants were exposed to short-term (two weeks) low temperature by the same method as in Chapter 3.

Flowers were marked 1, 3, 5 and 7 days after opening. The marked flowers were continuously exposed to low temperature, and very young pods and abscised flowers were sampled during the cooling treatment. The sampled pods and flowers were fixed in formalin-acetate-alcohol (FAA) solution and observed under a microscope with magnification $\times 30$. The pods with one or more fertilized ovules were counted as fertilized flowers, and the pods without fertilized ovules as unfertilized flowers. The rate of fertilization was calculated as follows:

The percentage of fertilized flowers (%) = (the number of fertilized flowers) / (the number of total flowers) $\times 100$.

Total flowers = (fertilized flowers) + (unfertilized flowers) + (abscised flowers)

In this analysis, since an error was assumed to follow a binomial distribution, a logit transformation was used to improve the homoscedasticity. The effect was tested by the likelihood ratio test. The procedure GENMOD of SAS was used for this calculation, too.

Results

Approximately 80 % of pollen grains were germinated in the treatment for 1 and 3 days (Fig. 4-1). However, germination rate was decreased to about 50 % in the treatment for 5 days. And the germination rates were lowered with the longer treatment. The rate was 20 % in the treatment for 11 days.

There is almost the same between Toyomusume and Hayahikari in anther length on the day when the first flower opened (Table 4-1). On the first day of the cooling treatment, the ratio of flowers which had more than 100 pollen grains on each stigma was 76 % in Toyomusume and 81 % in Hayahikari (Fig. 4-2). This ratio decreased with time during the cooling treatment rapidly in Toyomusume, but slowly in Hayahikari. The ratio of flowers with different pollen grain number was significantly different ($P=0.01$) between varieties (Table 4-2). On the seventh day of the cooling treatment, the ratio of flowers which had more than 100 pollen grains on each stigma was 22 % in Toyomusume, and 46 % in Hayahikari.

The percentage of fertilized flowers on the first day of the cooling treatment was near 100 % in both varieties, and it greatly decreased with time in Toyomusume, but only slightly in Hayahikari (Fig. 4-3). A reduction in the percentage of fertilized flowers on the seventh day was 89% in Toyomusume and only 32% in

Hayahikari. The difference was significant at the $P<0.01$ level (Table 4-3).

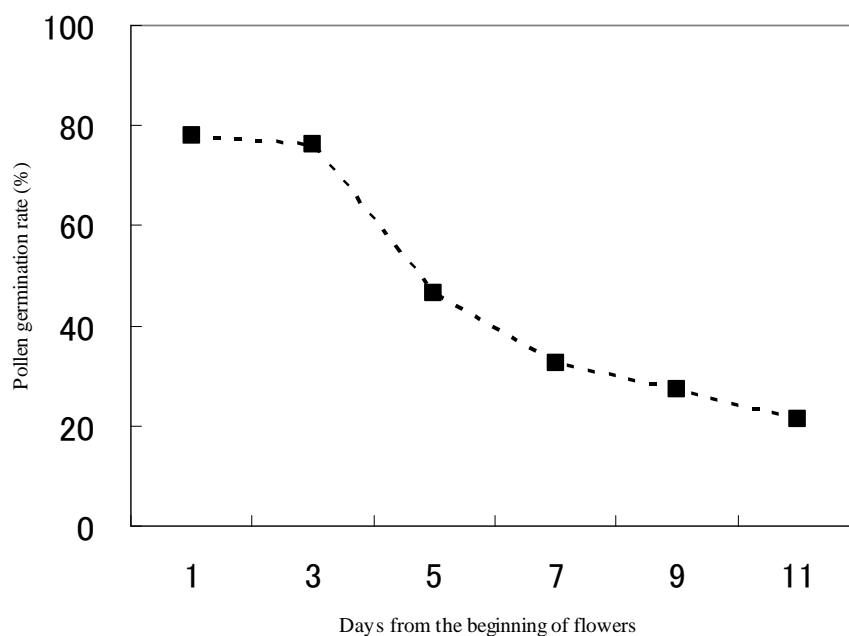


Fig.4-1. Effect of the cooling treatment on the pollen germination rate in Toyomusume. $n=8$.

Pollen germination rate (%) = (number of germinated pollen grains) / (total number of pollen grains) \times 100.

Table 4-1. Comparison of anther length in normal conditions.

Variety	Anther length (mm)
Toyomusume	0.17 ± 0.013
Hayahikari	0.17 ± 0.008

Data are means \pm standard errors.

Anther length was measured when the first flower opened.

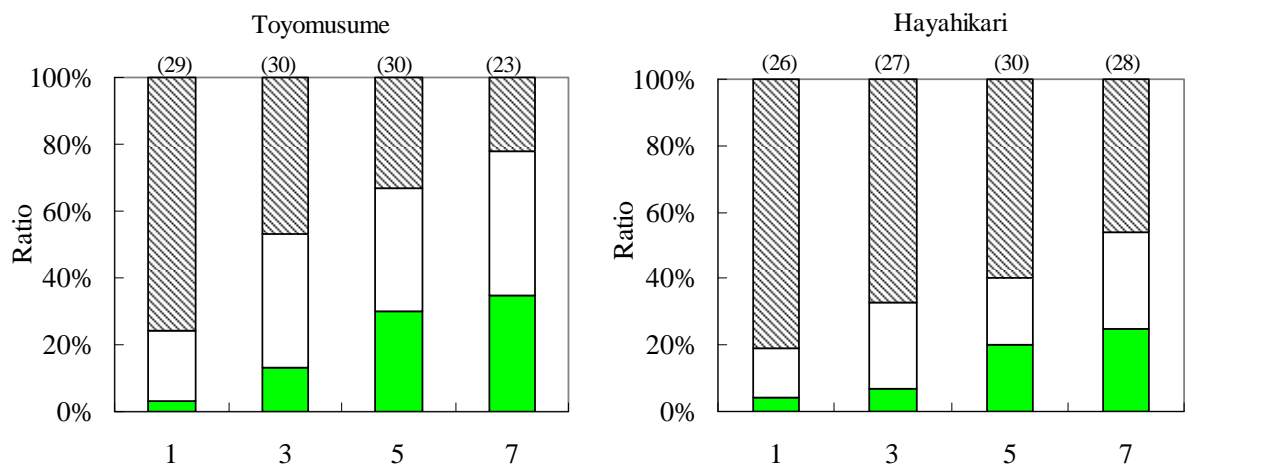


Fig. 4-2. Ratio of flowers classified by pollen number on stigma in the plants exposed to low temperature from the beginning of flowering.

(): Number of flowers examined

Pollen number ■ 0~50 □ 51~100 ▨ 101~

Table 4-2. Likelihood ratio test for the ratio of flowers classified by pollen number on stigma.

Source	Degrees of freedom	Chi Square	Probability
Variety	1	1.43	0.23
Category	2	48.38	<0.01
Variety × Category	2	9.20	0.01

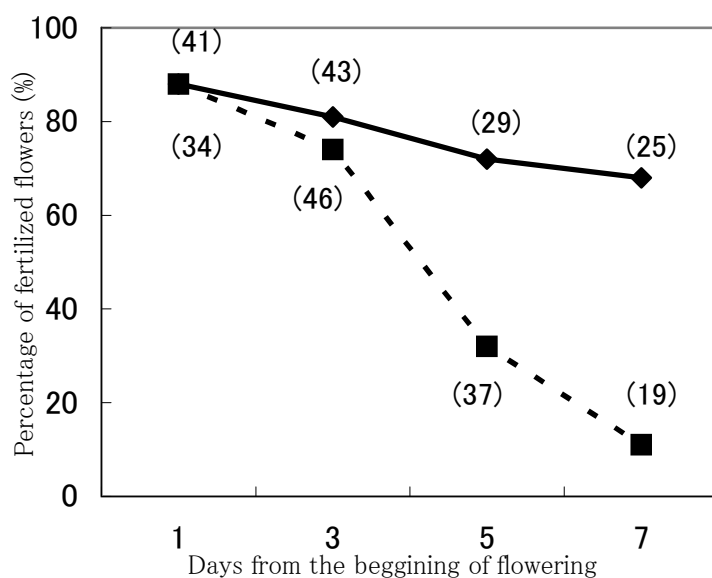


Fig. 4-3. Effect of the cooling treatment on the percentage of fertilized flowers.

Percentage of fertilized flowers(%)=(number of fertilized)/(number of total flowers)×100

—◆— Hayahikari -■- Toyomusume (): Number of examined flowers

Table 4-3. Likelihood ratio test
for the percentage of fertilized flowers.

Source	Degrees of freedom	Chi Square	Probability
Variety	1	17.57	<0.01
Days	1	75.82	<0.01

Discussion

When a flower which opens under low temperature, its organs sustain damage. In this chapter, three factors related to pod formation were investigated in a pistil.

Pollen-germination rates were affected by the cooling treatment. The rate was about 20 % in the treatment for 11 days. This result is consistent with the paper of Goto and Yamamoto (1972), who reported that pollen germination rate was decreased by cooling treatment for 15 days. However in this experiment, cooling treatment for only 5 days decreased the rate. This result indicates that pollen activity is affected by low temperature for a short time. Goto and Yamamoto (1972) did not investigate the effect of low temperature for a short time on pollen activity probably due to little reduction of pod number by cooling treatment for 5 days in their previous report (Saito et al, 1970).

Pollen germination rate in Hayahikari could not be investigated because Hayahikari produces cleistogamy flowers under low temperature. It is easy to find cleistogamy flowers on one or two days later after their flowering owing to a change of color in petals. On the contrary, it is hard to judge whether a cleistogamy flower opens or not, on the day when it opens. Further investigation on effect of low temperature on pollen activity in Hayahikari would clarify a varietal difference of sensitivity to low temperature in pollen. And some of the breeding materials which are cold-weather tolerance in the Tokachi Agricultural Experimental Station have a tendency to produce cleistogamy flowers. There may be a relationship between cleistogamy and cold-weather tolerance.

In Chapter 3, the pod-setting ability of flowers in the first week (F1W) was higher in Hayahikari than in Toyomusume. This suggests that the varietal difference in cold-weather tolerance is caused by the difference in the pollination and fertilization of flowers which were exposed to low temperature for one week.

The effect of low temperature on the fertilization is related to two floral organs. One is the low temperature tolerance of a stamen and the other is that of a pistil. In this experiment, the varietal difference in the low temperature tolerance of pistils was investigated, because Goto and Yamamoto (1972) reported that a stamen is more susceptible to low temperature than a pistil. And the reduction rate of ovules per pod due to low temperature was shown to be small in both Hayahikari and Toyomusume in Chapter 2. It was suspected that the varietal difference to low temperature in stamens would be existed.

In this chapter, pollination was reduced by cooling treatment in both varieties, and the effect of low temperature was significantly smaller in Hayahikari than in Toyomusume (Fig.4-2). Even if low temperature do damage to pollen grains to some extent, when sufficient number of the pollen grains pollinated on stigma, fertilization could be succeed. In other words, if pollen does not sustain damages, when a number of pollen deposited on stigma is very few in a flower, then it becomes the unfertilized flower. There are three factors which related to number of pollen deposited on a stigma; pollen number per anther, ability of anther

dehiscence and ability of pollen discharge. In rice plants, there is a high positive correlation between pollen number per anther and the length of anthers (Suzuki, 1981) and cold-weather tolerant varieties have longer anther length in normal conditions (Suzuki, 1982). In this experiment, the anther length of both varieties was nearly the same (Table 4-1). Given that there is a similar correlation between number of pollen and anther length in soybean too, there is not a large difference in pollen number per anther between two varieties. Pollen number per anther is not a cause of the varietal difference in cold- weather tolerance in soybeans.

These results indicate that low temperature caused a reduction in anther dehiscence or in pollen discharge, as a result, a number of pollen deposited on stigma was small, and there is a varietal difference to low temperature in pollination.

Furthermore, the number of pollen deposited on stigma was closely correlated with the percentage of fertilized flowers (Fig.4-3). Hayahikari showed a significantly higher percentage of fertilized flowers than Toyomusume. Since a close relationship was observed between the pollen number per stigma and the percentage of fertilized flowers, it can be concluded that the effect of low temperature on pollination are closely related with the fertilization of flowers. A superior fertilization ability at low temperature is one of the factors required in cold-weather tolerance.

Chapter 5 Analysis of correlation of cold-weather tolerance with agronomic traits for breeding.

The Tokachi Agricultural Experiment Station has been conducting soybean breeding to develop tolerance to cold-weather damage mainly due to poor growth and abscission of flowers and pods. Actual selection for poor growth is done in a field under mild low-temperature conditions throughout soybean growth, because damage due to low temperature in the early stage of growth can be sometimes masked by the high temperature during the subsequent growth period. On the other hand, the selection for abscission of flowers and pods is done using a phytotron kept at low temperature during the flowering stage. However, the field selection is not efficient in a hot summer year, and the selection in a phytotron limits the number of breeding materials due to the limited area of the phytotron. Therefore, if some plant characteristics related to cold-weather tolerance can be identified, a larger number of breeding materials can be screened.

Soybean cultivars with yellow seed coats are classified into two categories based on hilum color (yellow and brown) in Japan. Because Japanese consumers do not prefer the soybeans with a brown hilum, cold-weather tolerance has been improved mainly in yellow hilum soybeans in Hokkaido. Most of the tawny-pubescence soybeans (*T/T*) irrespective of hilum color (yellow: *I/I* or brown: *i-i/i-i*) are more tolerant to cold-weather than gray-pubescence soybeans (*t/t*) as observed in the progeny from many crosses between tawny pubescence with brown hilum soybeans (*T/T*, *i-i/i-i*) and gray pubescence with yellow hilum soybeans (*t/t*, *I/I*). Therefore, it has been assumed that *T/t* would contribute more to cold-weather tolerance than *I/i-i*. On the other hand, Tsuchiya et al. (1976) and Sanbuichi (1979) reported that growth vigor under low temperature conditions in the early stages of growth is correlated with cold-weather tolerance in soybeans. It was observed that lines with later flowering have superior growth vigor to lines with earlier flowering from some crosses, and hypothesized that flowering time was related to growth vigor.

In this chapter, to contribute to the breeding of yellow-hilum soybeans with cold-weather tolerance, the relationships between cold-weather tolerance and two characteristics, pubescence color and flowering time were investigated. First, the growth and yield of near isogenic lines for pubescence color (*T/t*) of yellow-hilum soybeans in relatively warm and cool regions were examined. Next, the pod number and seed yield of near isogenic lines for a combination of pubescence color (*T/t*) and flowering time (*E1/e1*) exposed to low temperature during the flowering period were examined. In addition, gray pubescence lines with different flowering times were selected from the crosses between early and late flowering lines, and their cold-weather tolerance was also examined.

Materials and Methods

Experiment 5-1. Correlation of the pubescence color with dry matter weight and seed yield in warm and cool regions.

Two pairs of near isogenic lines (NILs) for a pubescence color gene (*T* and *t*), which were developed at the Hokkaido Prefectural Tokachi Agricultural Experiment Station (Tokachi AES), were used. One pair was 0409*T* and 0409*t* and the other was 0518*T* and 0518*t*. 0409*T* and 0518*T* have tawny pubescence, yellow seed coat and hilum, and purple flowers (*T/T*, *I/I*, *r/r*, *O/O*, *W1/W1*; reviewed by Palmer and Kilen 1987). 0409*t* and 0518*t* have gray pubescence, yellow seed coat and hilum, and purple flowers (*t/t*, *I/I*, *r/r*, *O/O*, *W1/W1*). 0409*T/t* was derived from a cross between the landrace Okuhara-No.1 (*T/T*, *i-i/i-i*, *r/r*, *O/O*, *W1/W1*) and the breeding line Tokei No.809 (*t/t*, *I/I*, *r/r*, *O/O*, *W1/W1*) which was developed at the Tokachi AES. 0518*T/t* was derived from a cross between Tokei No.804 (*T/T*, *i-i/i-i*, *r/r*, *O/O*, *W1/W1*) and Tokei No.809 bred at the Tokachi AES. Each parent has a distant genetic background. Artificial crossings were made in 1992 (0409*T/t*) and in 1993 (0518*T/t*). Heterozygous plants for *T* and *t* were selected in six succeeding generations in 0409*T/t* and in seven succeeding generations in 0518*T/t*. Thus, the genetic identity of 0409*T* and *t* and that of 0518*T* and *t* are expected to be approximately 96 % and 97 %, respectively.

The growth and yield of these NILs were examined at Memuro which has relatively warm weather and in three cool regions [Kamishihoro (43° 14' N, 143° 18' E), Taiki (42° 31' N, 143° 31' E) and Kunneppu (43° 47' N, 143° 42' E)] in eastern Hokkaido. The experiments were conducted in 2001 at Memuro, Kamishihoro and Taiki, and in 2002 at Kunneppu, and all the experimental fields are dry Andosol. The average temperature during the growth period was 16.0°C at Memuro, 14.6°C at Kamishihoro, 13.8°C at Taiki and 15.2°C at Kunneppu. They were planted at the end of May at all locations. At each location, trials consisted of randomized blocks with four replications. Plots were comprised of two to four 300 cm rows 60 cm apart. Plants were thinned to a density of 16.7 plants m⁻². Fertilizer was applied according to Hokkaido fertilization standards for expected yield of 2.5t ha⁻¹ (0.2 N-1.8 P₂O₅-0.9 K₂O kg a⁻¹).

Dry matter weight of leaves, stems, roots and nodules were measured in 2001 trials. The sampling was conducted at the R1 stage (Fehr et al., 1971) at Memuro and Kamishihoro, and at the R3 stage at Taiki. At maturity in all trials, the number of pods and seed yield were measured, and were subjected to the t-test.

Experiment 5-2. Effect of low temperature during the flowering stage on the photosynthesis in NILs for pubescence color.

0518*T* and 0518*t*, one pairs of NILs for a pubescence color gene, were used. The experiment was conducted in 2001 at the Tokachi AES. On 1 May, 12 seeds of each line were planted in a pot (1/2000 a), filled with dry Andosol supplemented with a synthetic fertilizer (0.6 N- 5.3 P₂O₅-2.7 K₂O kg a⁻¹). Two weeks after seedling emergence, pots were thinned to two plants per pot. The plants were grown in an experimental facility having a plastic roof and no walls until the flowering time, then they were transferred to a phytotron. 18 plants of each line (9 pots) were grown in the experimental facility and 12 plants were subjected to a cooling treatment in a phytotron. The air temperature in the phytotron was set at 18/13 (day/night)°C and 15/10 (day/night)°C. The cooling treatment was applied for one week after the

beginning of flowering. The remaining 6 plants of each line were grown at 23/18 (day/night)°C, normal temperature.

Leaf photosynthesis was measured on the seventh day of the treatment and the control. A terminal leaflet of a third fully developed trifoliate leaf counted from main stem was selected for the photosynthesis measurement using a portable photosynthesis system (SHIMADZU: Type SPB-H3). The data were recorded on clear day and were subjected to the t-test.

Experiment 5-3. Effect of low temperature at the flowering stage on the number of pods and seed yield in NILs for a combination of pubescence color and flowering time.

NILs for a combination of pubescence color gene (*T* and *t*) and flowering time (*E1* and *e1*), *te*, *tE*, *Te* and *TE*, were developed from a cross between Tokei No.738 (*T/T*, *E1/E1*, *dt1/dt1*, *i-i/i-i*, *r/r*, *O/O*, *W1/W1*) and Tokei No.817 (*t/t*, *e1/e1*, *dt1/dt1*, *I/I*, *r/r*, *O/O*, *W1/W1*), which were developed at the Tokachi AES. Artificial crossing was conducted in 1993. Heterozygous plants for *T/t* and *E1/e1* were selected in five succeeding generations. Thus, the genetic identity between lines is expected to be approximately 94 %. Although *E7* is closely linked to *E1* (Cober and Voldeng 2001), the effect of *E7* on the NILs is unknown.

The characteristics of *te* are gray pubescence (*t*), early flowering (*e1*), determinate stem, yellow seed coat, brown hilum and purple flowers (*t/t*, *e1/e1*, *dt1/dt1*, *i-i/i-i*, *r/r*, *O/O*). Those of *tE* are gray pubescence (*t*), late flowering (*E1*), determinate stem, yellow seed coat, brown hilum and purple flowers (*t/t*, *E1/E1*, *dt1/dt1*, *i-i/i-i*, *r/r*, *O/O*, *W1/W1*). Those of *Te* are tawny pubescence (*T*), early flowering (*e1*), determinate stem, yellow seed coat, brown hilum and purple flowers (*T/T*, *e1/e1*, *dt1/dt1*, *i-i/i-i*, *r/r*, *O/O*, *W1/W1*). Those of *TE* are tawny pubescence (*T*), late flowering (*E1*), determinate stem, yellow seed coat, brown hilum and purple flowers (*T/T*, *E1/E1*, *dt1/dt1*, *i-i/i-i*, *r/r*, *O/O*, *W1/W1*).

The experiment was conducted from May through October 2000 at the Tokachi AES. On 25 May, 12 seeds of each line were planted in a pot (1/2000 a), filled with dry Andosol supplemented with a synthetic fertilizer (0.6 N- 5.3 P₂O₅-2.7 K₂O kg a⁻¹). Two weeks after seedling emergence, pots were thinned to two plants per pot. The plants were grown in an experimental facility having a plastic roof and no walls until the flowering time, then they were moved to a phytotron and grown there until maturity. Twenty plants of each line (10 pots) were grown in the experimental facility and 10 plants were subjected to a cooling treatment in a phytotron. The air temperature in the phytotron was set at 18/13 (day/night)°C, the same as in Chapter 2. The cooling treatment was applied for four weeks after the beginning of flowering. The plants were shaded (50 % shade) during the cooling treatment. After the cooling treatment, the plants were grown at 23/18 (day/night)°C. The remaining 10 plants of each line were used as the control, which were grown at 23/18 (day/night)°C from flowering to maturity.

Pots were randomized in the phytotron and the experimental facility, and repositioned at least once a week. At the maturity, the number of main stem nodes, the number of pods and the weight of seeds were measured for each plant. Cool tolerance index (CTI) was calculated for the above data to evaluate a rate of reduction by the cooling treatment, according to Sanbuichi (1979) as follows;

$$\text{CTI} = (\text{the value in the cooling treatment}) / (\text{the value in the control}) \times 100.$$

The data were subjected to the analysis of variance.

Experiment 5-4. Selection experiment in the groups with different flowering times in gray pubescence soybeans.

The cross between the landrace Konsen-noshi (early flowering) and the breeding line Tokei No. 809 (late flowering), which was named Tokou 0636, and the cross between the landrace Okuhara No. 1 (early) and the breeding line Tokei No. 809 (late), which was named Tokou 0409, were used to select early, mid and late flowering groups. Konsen-noshi has tawny pubescence, early flowering, determinate stem, yellow seed coat, brown hilum and purple flowers (*T/T*, *dt1/dt1*, *i-i/i-i*, *r/r*, *O/O*, *W1/W1*, maturity genes are unknown). Tokei No.809 has gray pubescence, late flowering, determinate stem, yellow seed coat, yellow hilum and purple flowers (*t/t*, *dt1/dt1*, *I/I*, *r/r*, *O/O*, *W1/W1*, maturity genes are unknown). Okuhara No.1 has tawny pubescence, early flowering, determinate stem, yellow seed coat, brown hilum and purple flowers (*T/T*, *dt1/dt1*, *i-i/i-i*, *r/r*, *O/O*, *W1/W1*, maturity genes are unknown). Soybean plants with different flowering times from the plants with gray pubescence and yellow hilum in both crosses were selected. Selection procedures are as follows.

Artificial crossings were conducted in 1992 (Tokou 0409) and in 1994 (Tokou 0636). The bulk method was used from F_2 to F_4 , and then the pedigree method was conducted in F_5 . About 200 gray pubescence plants were randomly harvested in F_2 and F_3 , brown hilum plants were discarded after threshing, and 100 plants were randomly selected for the next generation. Three groups differing in the flowering time were selected in F_4 (15 plants per group) and F_5 (four lines per group). The first group, with the early flowering time similar to Konsen-noshi and Okuhara No.1, was named 'Group E', the second group with the late flowering time similar to Tokei No.809, 'Group L', and the third group with the mean flowering time of both parents (mid-flowering), 'Group M'. 30 plants from each line of F_5 were planted, and the lines with no segregation related to the flowering time were finally selected.

Cooling treatment was conducted for F_6 plants in 1998. On 30 April, 14 seeds were planted per pot (25 l) filled with dry andosol soil supplemented with a synthetic fertilizer ($0.6 \text{ N}-5.3 \text{ P}_2\text{O}_5-2.7 \text{ K}_2\text{O kg a}^{-1}$) and they were thinned to five per pot, two weeks after emergence. Four pots were tested per line.

The plants were grown in an experimental facility under a plastic roof with walls, except during the cooling treatment. The average daily temperature for every ten days in the experimental period is shown in Fig.5-1. The cooling treatment was applied in the same way as in Experiment 5-3. At maturity, the number of main stem nodes, the number of pods and seed yield were measured. CTI was calculated in the same way as in Experiment 5-3, and subjected to the analysis of variance between groups.

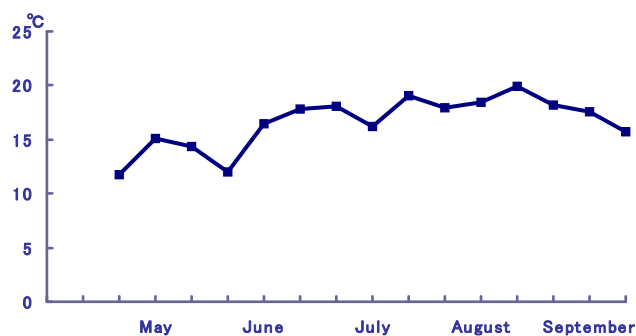


Fig.5-1. Average daily temperature every 10 days during soybean growth in 1998. Arrows indicate soybean growth stage.

— Average Temperature

Results

Experiment 5-1. Correlation of the pubescence color with dry matter weight and seed yield in warm and cool regions.

At Memuro which has a relatively warm weather for eastern Hokkaido, little difference in the dry weight of the leaves, stems, roots and nodules at the flowering stage were observed between the NILs for tawny pubescence (*T*) and gray pubescence (*t*) in both pairs, 0409 *T/t* and 0518 *T/t* (Fig. 5-2 and Fig. 5-3). On the other hand, in the two cool regions (Kamishihoro and Taiki), the dry weight of leaves, stems, roots and nodules of the tawny lines were heavier than those in the gray lines. At Kamishihoro, 0409*T* was 117% of 0409*t* and 0518*T* was 109 % of 0518*t* in leaves. 0409*T* was 124% of 0409*t*, 0518*T* was 110 % of 0518*t* in stems, and 0409*T* was 125% of 0409*t* in roots, and 0409*T* was 116% of 0409*t*, 0518*T* was 108 % of 0518*t* in nodules. And at Taiki, 0409*T* was 109 % of 0409*t* and 0518*T* was 111 % of 0518*t* in leaves. 0409*T* was 112% of 0409*t*, 0518*T* was 110 % of 0518*t* in stems, and 0409*T* was 168 % of 0409*t*, 0518*T* was 166 % of 0518*t* in nodules. The dry weight of nodules at Taiki was lighter than that at other locations in both tawny and gray lines. Dry weight of leaves, stems and roots at Taiki were generally heavier than those at Kamishihoro, because the plants were sampled at the R3 stage at Taiki but at the R1 stage at Kamishihoro.

The seed yield had a positive correlation with the average temperature during the soybean growth (Table 5-1). It was the highest at Memuro (mean temperature was 16.0 °C) followed by Kunneppu (15.2°C), Kamishihoro (14.6°C) and Taiki (13.8°C) in this order, in both 0409 *T/t* and 0518 *T/t*. At Memuro, the number of pods and the seed yield were not different between the tawny and gray lines in both pairs. However, there were significant differences between the tawny and gray lines in each pair at Kamishihoro and Taiki. At Kamishihoro, 0409*T* were 117% in the number of pods and 116 % of 0409*t* in the seed yield, while 0518*T* were 128 % and 117 % of 0518*t*, respectively. At Taiki, 0409*T* were 127% in the number of pods and 132 % of 0409*t* in the seed yield, and 0518*T* were 115 % and 119 % of 0518*t*, respectively. A similar tendency was found at Kunneppu. 0409*T* was 107% in the number of pods and 111 % of 0409*t* in the seed yield, while 0518*T* was 110 % and 118 % of 0518*t*, respectively. The lineal differences were significant in the number of pods for 0518 *T/t*, and in the seed yield for both pairs.

Table 5-1. Pod number and seed yield at the maturity of NILs for gray-pubescence line (0409*t* and 0518*t*) and for tawny-pubescence line (0409*T* and 0518*T*) grown at different locations.

Year	Location	Average temperature during growth	(Lines) NILs	Planting date	Maturity date	Pods per plant	Seed yield (kg/a)
2001	Memuro	16.0 °C	0409 <i>t</i>	21,May	5,October	61.8 (100)	26.5 (100) ns
			0409 <i>T</i>	Ditto	4,October	60.7 (98)	26.4 (100) ns
			0518 <i>t</i>	Ditto	8,October	62.4 (100)	28.1 (100) ns
			0518 <i>T</i>	Ditto	7,October	65.7 (105)	27.3 (97) ns
	Kamishihoro	14.6 °C	0409 <i>t</i>	25,May	22,October	41.4 (100)	10.2 (100) **
			0409 <i>T</i>	Ditto	21,October	48.4 (117)	11.8 (116)
			0518 <i>t</i>	Ditto	23,October	41.5 (100)	13.2 (100) *
			0518 <i>T</i>	Ditto	22,October	53.0 (128)	15.4 (117)
	Taiki	13.8 °C	0409 <i>t</i>	29,May	23,October	41.6 (100)	11.6 (100) *
			0409 <i>T</i>	Ditto	22,October	52.7 (127)	15.3 (132)
			0518 <i>t</i>	Ditto	24,October	39.6 (100)	11.5 (100) *
			0518 <i>T</i>	Ditto	21,October	45.5 (115)	13.7 (119)
2002	Kunneppu	15.2 °C	0409 <i>t</i>	22,May	9,October	58.1 (100)	21.0 (100) *
			0409 <i>T</i>	Ditto	9,October	62.1 (107)	23.3 (111) ns
			0518 <i>t</i>	Ditto	9,October	57.6 (100)	23.9 (100) *
			0518 <i>T</i>	Ditto	9,October	63.3 (110)	28.3 (118) **

*,** and ns indicate significant at P=0.05, P=0.01 and not significant difference, respectively.

Values in parentheses indicate relative percentages of the values in the *T* (tawny pubescence) line to those in the corresponding *t* (gray pubescence) line.

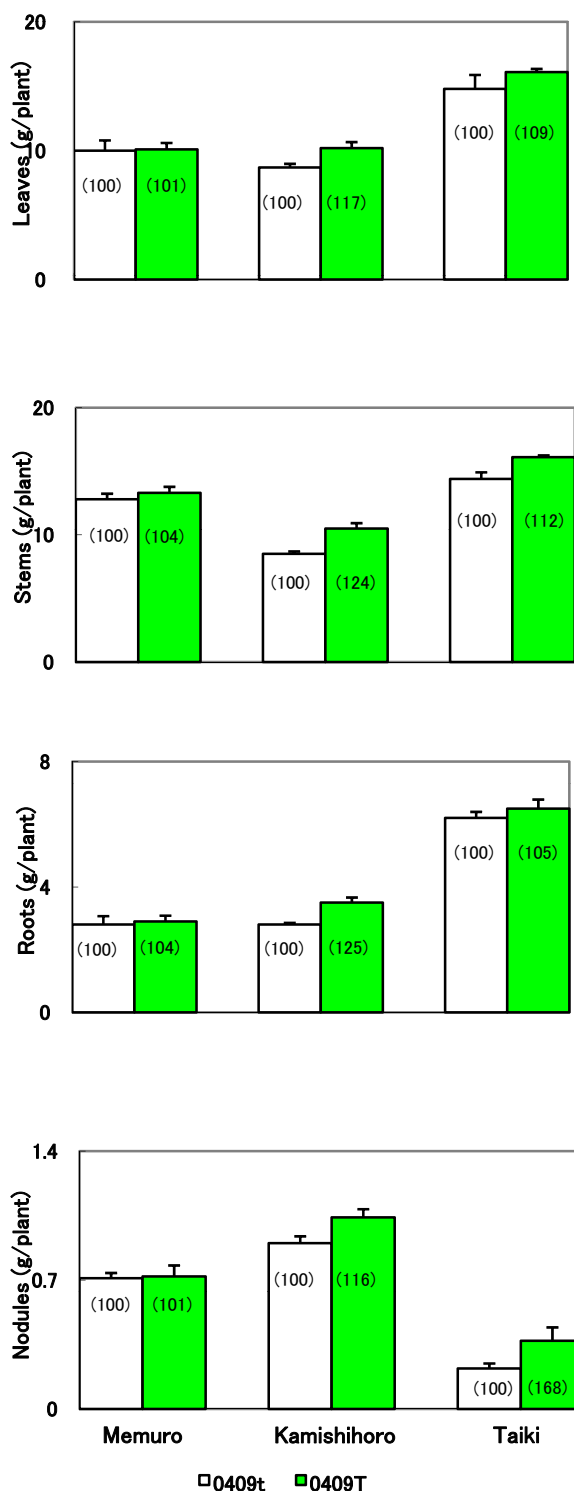


Fig.5-2. Dry matter weight of leaves, stems, roots or nodules at the flowering stage in 0409t and 0409T in warm (Memuro) and cool regions (Kamishihoro and Taiki). Values in parentheses indicate relative percentages to each t (gray pubescence) line. Error bars represent standard errors of the mean.

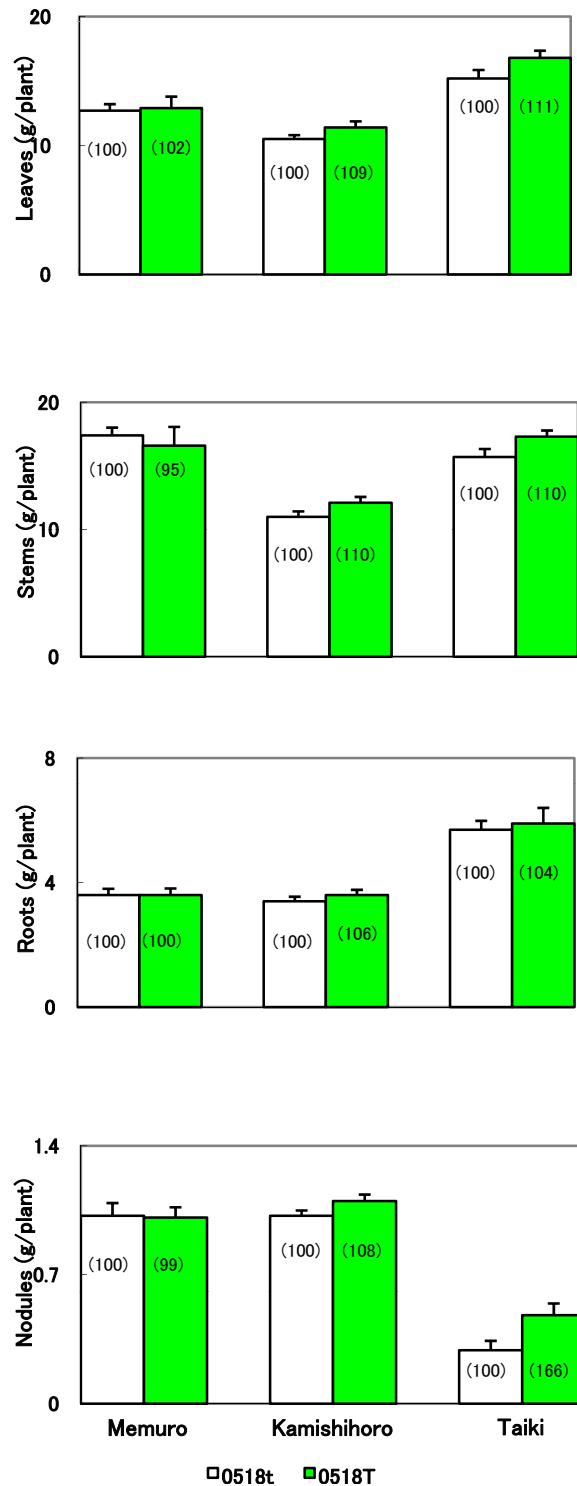


Fig.5-3. Dry matter weight of leaves, stems, roots or nodules at the flowering stage in 0518t and 0518T in warm (Memuro) and cool regions (Kamishihoro and Taiki). Values in parentheses indicate relative percentages to each t (gray pubescence) line. Error bars represent standard errors of the mean.

Experiment 5-2. Effect of low temperature during the flowering stage on the photosynthesis in NILs for pubescence color.

The leaf photosynthesis had a positive correlation with temperature in both 0518*t* and 0518*T* (Table 5-2). Compared to the normal condition (23/18 °C), there were reductions in the cooling treatments (18/13 °C and 15/10 °C) in both lines. The leaf photosynthesis was higher in 0518*T* than in 0518*t* in the cooling treatment. 0518*T* were 148 % at 18/13 °C and 121 % at 15/10 °C of 0518*t*, and the lineal difference was significant at 18/13 °C.

Table 5-2. Photosynthetic rates of 0518*T/t* at different temperatures (day/night).

	Photosynthetic rates($\mu\text{molCO}_2\text{m}^{-2}\text{S}^{-1}$)		
	23/18°C	18/13°C	15/10°C
0518 <i>t</i>	12.1(100)	6.5(100)	2.9(100)
0518 <i>T</i>	13.5(112)	9.6(148)	3.5(121)
t-test results	ns	**	ns

Values in parentheses indicate relative percentages of the values in the *T* (tawny pubescence) line to those in the *t* (gray pubescence) line.

** and ns indicate significant at $P = 0.01$ and not significant difference, respectively.

Experiment 5-3. Effect of low temperature during the flowering stage on the number of pods and seed yield in NILs for a combination of pubescence color and flowering time.

The genetic effect of the maturity gene (*E1/e1*) on flowering time, maturity, the number of main stem nodes, the number of pods and the seed yield in the control were greater than those of the pubescence color gene (*T/t*). The *e1* lines (*te* and *Te*) flowered and matured almost 10 days earlier than the *E1* lines (*tE* and *TE*) in the control conditions (Table 5-3). The numbers of main stem nodes and pods were smaller in the *e1* lines (*te* and *Te*) than in the *E1* lines (*tE* and *TE*) in the control. The numbers of main stem nodes in the control in *te*, *tE*, *Te* and *TE* were 7.2, 11.5, 7.1 and 10.8, the numbers of pods were 19.9, 25.4, 19.4 and 27.6, and the seed yields were 12.0, 12.8, 12.3 and 15.5, respectively.

No significant differences were found in the number of main stem nodes to the cooling treatment (Table 5-4). However, the effect of the cooling treatment on number of pods and seed yield depended on the lines. In the early flowering lines, the reduction rates of number of pods and seed yield for the tawny pubescence line were small, but those for the gray pubescence line were large. For both tawny and gray pubescence lines with late flowering, they were small. The cooling treatment \times *e1* versus *E1* within *t* interaction was significant, but the cooling treatment \times *e1* versus *E1* within *T* interaction was not. The number of pods was reduced to 73 % of the control in *te*, whereas *tE*, *Te* and *TE* were reduced only to 96 %, 98 % and 98 %, respectively. Although *te* was 39 % of the control, *tE*, *Te* and *TE* were 77 %, 65 % and 77 %, respectively in the seed yield.

Table 5-3. Effect of cooling treatment during flowering on the number of main stem nodes, pod number and seed yield at maturity, in NILs for a combination of pubescence color (*t* and *T*) and flowering time (*eI* and *El*).

Line	Treatment	Flowering date	Maturity date	No. of main stem nodes	CTI	Pods per plant	CTI	Seed yield (g / plant)	CTI
<i>te</i>	Control	5, July	21, Sep	7.2	108	19.9	73	12.0	39
	Cooling	5, July	28, Sep	7.8		14.5		4.7	
<i>tE</i>	Control	14, July	28, Sep	11.5	104	25.4	96	12.8	77
	Cooling	14, July	5, Oct	12.0		24.3		9.8	
<i>Te</i>	Control	5, July	10, Sep	7.1	109	19.4	98	12.3	65
	Cooling	5, July	25, Sep	8.4		19.0		8.0	
<i>TE</i>	Control	14, July	20, Sep	10.8	107	27.6	98	15.5	77
	Cooling	14, July	28, Sep	11.6		27.1		11.8	

CTI (Cool tolerance index) = (value in the cooling treatment) / (value in the control) × 100.

te : gray pubescence with early flowering, *tE* : gray pubescence with late flowering,

Te: tawny pubescence with early flowering, *TE*: tawny pubescence with late flowering.

Table 5-4. Effect of cooling treatment on NILs for a combination of pubescence color (*t* and *T*) and flowering time (*eI* and *El*): analysis of variance results.

	Main stem nodes per plant	Pods per plant	Seed yield per plant
Cooling treatment	ns	**	**
Cooling treatment × <i>t</i> versus <i>T</i>	ns	ns	ns
Cooling treatment × <i>eI</i> versus <i>El</i> within <i>t</i>	ns	*	**
Cooling treatment × <i>eI</i> versus <i>El</i> within <i>T</i>	ns	ns	ns

t : gray pubescence, *T*: tawny pubescence, *eI*: early flowering, *El*: late flowering.

*, ** and ns indicate significant at P = 0.05, P = 0.01 and not significant difference, respectively.

Experiment 5-4. Selection experiment in the groups with different flowering times in gray pubescence soybeans

Selection effectiveness with respect to the flowering time was observed. In the control conditions, the flowering date was 17 June in the early flowering group E of both Tokou 0636 and Tokou 0409. It was 20 and 21 June in the mid flowering group M of Tokou 0636 and Tokou 0409, respectively, and 24 and 26 June in the late flowering group L of Tokou 0636 and Tokou 0409, respectively (Table 5-5).

The number of main stem nodes, the number of pods and the seed yield in the control condition were greater in the late flowering group than in the early flowering group. The effect of the cooling treatment on the number of pods and seed yield was the largest (CTIs were the lowest) in group E followed by group M

and group L in both Tokou 0636 and Tokou 0409. The numbers of pods in the cooling treatment were 34 % of the control for Group E, 52 % for Group M and 82 % for Group L in Tokou 0636, and they were 53 %, 88 % and 97 %, respectively in Tokou 0409. The seed yield in the treatment was 20 % of the control for Group E, 33 % for Group M and 59 % for Group L in Tokou 0636, and they were 29 %, 56 % and 65 %, respectively in Tokou 0409. The difference between the groups is significant (Table 5-5).

Similar effect of the cooling treatment was observed in the parent plants with different flowering times (Table 5-6). The CTIs of Konsen-Noshi and Okuhara No. 1 (tawny pubescence and early flowering) and Tokei No. 809 (gray pubescence and late flowering) for the number of pods and the seed yield were roughly the same. These results are coincident with Experiment 5-3, in which the CTIs of *Te* (tawny pubescence and early flowering) and *tE* (gray pubescence and late flowering) lines were approximately the same, and their CTIs were as high as CTI of a cold-weather tolerant variety.

Table 5-5. Effect of cooling treatment on the number of main stem nodes, pod number and seed yield per plant in the groups with different flowering times.

Crossing	Group	Treatment	Flowering	Maturity	No. of main stem nodes	Pods		Seed		
			Date	date		CTI	plant	CTI	(g/plant)	
Tokou 0636	E	Control	17, June	28,Aug	8.3	96	13.0	34	8.2	20
		Cooling	17, June	18,Sep	8.0		4.4		1.6	
	M	Control	20, June	8,Sep	10.2	93	18.0	52	10.2	33
		Cooling	20, June	19,Sep	9.5		9.4		3.4	
	L	Control	24, June	10,Sep	10.5	96	19.3	82	11.8	59
		Cooling	24, June	27,Sep	10.1		15.9		7.0	
Analysis of Variance						ns		**		**
Tokou 0409	E	Control	17, June	17,Aug	9.2	95	11.6	53	4.5	29
		Cooling	17, June	9,Sep	8.7		6.2		1.3	
	M	Control	21, June	1,Sep	10.7	92	15.3	88	10.0	56
		Cooling	22, June	15,Sep	9.8		13.5		5.6	
	L	Control	26, June	17,Sep	12.2	93	17.2	97	10.3	65
		Cooling	26, June	30,Sep	11.4		16.7		6.7	
Analysis of Variance						ns		*		*

CTI (Cool tolerance index) =(value in the cooling treatment) / (value in the control)× 100.

*, ** and ns indicate significant at P=0.05, P=0.01 and not significant difference between groups, respectively.

Tokou 0636: Konsen-noshi×Tokei No.809, Tokou 0409: Okuhara No.1×Tokei No.809.

Group E: Early flowering group, Group M: Mid flowering group, Group L: Late flowering group.

Table 5-6. Effect of cooling treatment on the number of main stem nodes, pod number and seed yield at maturity in the parent varieties.

Variety	Treatment	Flowering	Maturity	No. of main stem nodes	CTI	Pods per		Seed Yield	
		date	date			plant	CTI	(g/plant)	CTI
Konsen-Noshi (early flowering)	Control	15, June	24, Aug	9.0	92	16.3	80	9.6	50
	Cooling	15, June	14, Sep	8.3		13.0		4.8	
Okuhara No 1 (early flowering)	Control	16, June	24, Aug	9.8	90	17.3	84	9.8	54
	Cooling	16, June	22, Sep	8.8		14.5		5.3	
Tokei No. 809 (late flowering)	Control	26, June	12, Sep	12.5	94	20.3	81	14.5	55
	Cooling	26, June	1, Oct	11.8		16.5		8.0	
Toyomusume	Control	20, June	8, Sep	9.2	99	19.0	38	12.2	25
	Cooling	20, June	22, Sep	9.1		7.3		3.1	
Kitamusume	Control	22, June	5, Sep	10.6	102	19.9	87	12.5	58
	Cooling	22, June	22, Sep	10.8		17.4		7.3	

CTI (Cool tolerance index) =(value in the cooling treatment) / (value in the control)×100.

Toyomusume:cold- weather sensitive variety, Kitamusume:cold-weather tolerant variety.

Discussion

Relation between pubescence color and tolerance to cold-weather damage.

A multiple allele locus *I/i-i/i-k/i* controls the distribution of color of seed coat and hilum (Palmer and Kilen, 1987). In brown hilum soybeans (*i-i/i-i*), coloration is restricted to the hilum, whereas coloration is completely suppressed in yellow hilum soybeans (*I/I*). Meanwhile, the pubescence color is controlled by a single locus, *T* (tawny) and *t* (gray) (Woodworth, 1921). All of the brown hilum soybeans, which have been developed in Hokkaido, have tawny pubescence (*T/T*, *i-i/i-i*), and while, yellow hilum soybeans commonly have gray pubescence (*t/t*, *I/I*). It has been observed that soybeans with tawny pubescence and brown hilum (*T/T*, *i-i/i-i*) are more tolerant to cold-weather damage than soybeans with gray pubescence and yellow hilum (*t/t*, *I/I*) in Hokkaido (Yumoto et al., 1995).

In warm region (Memuro), the number of pods and seed yield in NILs for tawny pubescence (tawny line) were similar to those in NILs for gray pubescence (gray line), but in cooler regions (Kamishihoro, Taiki and Kunneppu), those in tawny lines were significantly larger than those in gray lines. This indicates that the *T* allele is related to the tolerance to cold-weather damage. This finding is supported by Morrison et al. (1994), who found that yields of tawny varieties and lines were higher than those of gray ones in cool regions. It was also demonstrated that the gene *T* was effective for cold-weather tolerance in yellow hilum soybeans. The difference in seed yield related to *T/t* in the cooler regions can be partially attributed to the difference in growth vigor. Tsuchiya et al. (1976) and Sanbuichi (1979) found a relation between growth vigor during the low temperature and yield. Soybeans with greater growth vigor have higher yields than those with inferior growth vigor. In this experiment, the dry weight of the leaves, stems and nodules in tawny lines at the flowering stage were greater than those in gray lines in the cooler regions. Thus, it can be considered that the gene *T* is related to growth vigor during low temperature, and greater growth vigor

in the tawny lines than the gray lines is a cause of higher seed yield in cool conditions.

This difference can be partially explained by leaf photosynthesis. The leaf photosynthesis was higher in the tawny line than in the gray line under low temperature. Nielsen et al. (1984) found that increased shortwave radiation reflectance resulted in a lower canopy temperature in soybeans. It was speculated that gray pubescence may increase the shortwave radiation reflected from the canopy, thereby decreasing canopy temperature (Morrison et al., 1994). Marrowitch et al. (1986) reported that plant temperature has a relation with the rates of photosynthesis in soybeans. Therefore, under low temperature conditions, soybeans with gray pubescence would have been at a disadvantage and those with tawny pubescence favored.

Gene *T* is involved in flavonoid biosynthesis, and encodes a flavonoid 3'-hydroxylase (F3'-H) which hydroxylates the 3' position of a flavonoid (Buttery and Buzzell, 1973). A single base-deletion in the F3'-H gene is associated with gene *t* (Toda et al., 2002). A flavonoid with hydroxy configuration at the 3' position has a high level of antioxidant activity relative to that without it (Pratt, 1976). Antioxidant activity has been suggested to play an important role in cold-weather tolerance in maize (Prasad et al., 1994; Hodges et al., 1997; Pinhero et al., 1997) and in rice (Saruyama and Tanida, 1995; Sato et al., 2001). Funatsuki et al. (2002) showed that isozymes of an antioxidant enzyme is related to cold-weather tolerance in soybeans. The difference for growth vigor in cooler regions by pubescence color might be due to flavonoids which have antioxidant activity that reduces damage by low temperature in tawny soybeans.

Relation between the flowering time and the damage due to low temperature during the flowering stage

So far, eight loci have been reported to control the time of flowering and maturity in soybeans; *E1* and *E2* (Bernald, 1971), *E3* (Buzzell, 1971), *E4* (Buzzell and Voldeng, 1980), *E5* (McBlain and Bernard, 1987), *E6* (Bonato and Vello, 1999), *E7* (Cober and Voldeng, 2001) and *J* (Ray et al., 1995). Among them, *E1* has the greatest effect on the time of flowering and maturity (McBlain et al., 1987). However, the relation between flowering time and cold-weather tolerance has not been clarified yet.

The CTIs for the number of pods and the seed yield in tawny lines with early and late flowering time (*Te* and *TE*) were high in Experiment 5-3. This supports the theory that cold-weather tolerance of tawny soybeans is greatly related to the gene *T*. CTIs for the number of pods and the seed yield in gray lines with early flowering (*te*) were lower than those in tawny lines with late flowering (*Te*), but those in late flowering lines with gray pubescence (*tE*) were as high as those with tawny pubescence (*TE*). This result indicates that the flowering time might be related to the tolerance to damage due to low temperature in the flowering stage. To verify this presumption, the groups with different flowering times from two crosses (Tokou 0636 and Tokou 0409), which have gray pubescence and yellow hilum, were selected and the selected groups were exposed to low temperature.

As a result, the CTIs for the number of pods and seed yield in the later flowering group were significantly higher than those in the earlier flowering group in both crosses. This verified that the flowering time is related to the tolerance to damage due to low temperature in the flowering stage, and that late flowering soybeans have higher tolerance than early flowering soybeans.

Late flowering soybeans with high cold-weather tolerance seem to have a higher rate of nitrogen and

carbon assimilation than early flowering ones due to larger canopy. In addition, there is a positive correlation between flowering time and numbers of nodes and flowers per plant (Yumoto and Matsukawa, 1992). In Experiments 5-3 and 5-4, the lines and groups with later flowering had a larger number of main stem nodes, and they were expected to have a larger number of flowers than those with earlier flowering. When flowers of soybean are damaged by low temperature at the flowering stage, the resulting decrease in pod setting is compensated by flowers that open after the low-temperature period, and the degree of compensation depends on the number of flowers which are not damaged. Soybeans that have a larger number of total flowers open more flowers in a given period than soybeans with a smaller number of total flowers (Sanbuichi, 1964). Therefore, the degree of the above compensation in late flowering soybeans which have a larger number of total flowers is higher than in early flowering soybeans with fewer total flowers, because the former develops a greater number of flowers after the low-temperature period.

Judging from the magnitude of the effects of maturity genes which have been identified so far, it is possible to think that group E is *e1/e1* and group L is *E1/E1* in Experiment 5-3. Since the CTI in the seed yield in the *tE* was higher than that in the *te*, there is a possibility that the *E1* allele or closely linked gene is related to cold-weather tolerance. At the same time, the CTI in the seed yield was high in both *Te* and *TE*, and Weiss (1970) reported that the genes *T* and *E1* are closely linked. Assuming that the cold-weather tolerant gene is not the gene *T* itself but a closely linked gene(s), the gene(s) related to cold-weather tolerance might be identical one(s) which is closely linked with both *T* and *E1*. This is an area for future research.

In conclusion of this chapter, selecting yellow-hilm soybeans with tawny pubescence and later flowering time are both helpful for developing yellow-hilum soybeans cold-weather tolerance.

Chapter 6 Comprehensive discussions

Poor growth, abscission of flowers and pods, insufficient grain filling are the three major factors reducing soybean seed yields as affected by low temperature in Hokkaido (Yamamoto and Narikawa, 1966). The most common damage by low temperature is poor growth, but abscission of flowers and pods causes the most severe damage in soybean yield. Although breeding is the best countermeasure for cold-weather damage in soybean, researches which contribute to breeding for alleviation from the damage due to flower and pod abscission have been very limited.

In this dissertation, to analyze the factors controlling cold-weather tolerance in terms of plant physiology for soybean breeding, the following experiments were conducted. The factors controlling soybean growth are analyzed based on two main environmental factors in regard to cold-weather damage due to low temperature and less sunshine. Response of pod setting to cold weather among varieties under low temperature was investigated, and mechanisms of the tolerance in pollination and fertilization were attempted to be clarified. In order to develop a cold-weather tolerant variety by a simple method, relations between the cold-weather tolerance and two agronomic characteristics were analyzed by employing near-isogenic lines. The results of these experiments and ideas of the application to soybean breeding in Hokkaido are as follows.

The effects of low temperature and shading at the flowering stage on soybean growth were examined using two varieties of soybeans such as Hayahikari, (an excellent cold-weather tolerant variety) and Toyomusume, (a cold-weather sensitive variety) (Chapter 2). Effects of cooling and shading treatments on the vegetative growth were small in either variety. The reproductive growth was affected by low temperature and shading in both varieties. Low temperature at the flowering stage markedly reduced seed yield, and shading during the same period declined pod number. Cold-weather damage is mainly caused by low air temperature. A varietal difference in the seed yield after the cooling treatment is attributed to the difference in the response of all the yield components, especially the number of pods per plant, to the low temperature.

Cold-weather tolerance of all the yield components in Hayahikari is superior to those in Toyomusume. In the case of enhancement of cold-weather tolerance to sensitive varieties with other excellent characteristics, improving cold-weather tolerance of each yield component should be considered. Apparently, the most important element is the tolerance of pod setting at low temperature. However, a great varietal difference was also observed in grain size in this experiment. Before discussing the pod setting ability at low temperature, attention to cold-weather tolerance of grain size is drawn. With regard to grain size in this case, there are three kinds of factors; 1) a growing ability of fertilized ovules, 2) amount of a vegetative growth for grain filling, and 3) grain filling ability itself. The vegetative growth rate was not affected by cooling treatment in this experiment. It can be considered that a growing ability of fertilized ovules or grain filling ability was damaged. Either factor has a close relation with growth after cooling treatment. Low temperature causes continuing damage to sensitive soybeans not only in cooling treatment but also after it. Takahashi and Asanuma (1996) reported that yield of a tawny pubescence isogenic line is higher due to greater grain size in the cooling treatment at the flowering stage than a gray pubescence one, the gene *T* is related to greater grain size after cooling treatment. These results suggest that introduction of the gene *T* is one of the measures against a decrease of grain size owing to low temperature.

The total pod setting ability of Hayahikari for low temperature was higher than that of Toyomusume, but both varieties showed the same susceptibility to shading. Cold-weather tolerant varieties such as Hayahikari have shown good performance in most years with cold weather damage. However, in the years with extremely severe damage, they may also receive some damage. Therefore, it is important to develop new types of tolerance. Cold-weather damage is caused by insufficient sunlight as well as low temperature. Enhancement of tolerance to shading in soybeans is a key factor to improve cold-weather tolerance. Improving shading tolerance will help stabilize soybean production in soybean fields on coast area, one of the cool regions, have always less sunshine due to sea fog, too. So far, cold-weather tolerance has been studied only from the angle of low temperature, and tolerance to low temperature has been checked on lots of genetic resources using a phytotron. However, tolerance to shading has not been screened. What is important are to find soybeans which are tolerant to shading and to investigate an interaction between low temperature and shading more. The results indicate that breed of a variety which has a superior pod setting ability not only at low temperature but also under insufficient sunlight would be an effective way to progress cold-weather tolerance.

The effects of short-term exposure to low temperature and long-term exposure from the beginning of flowering on pod setting were examined in Hayahikari and Toyomusume (Chapter 3). The short-term exposure to low temperature did not decrease the total number of pods even in Toyomusume. However, pod setting ability during exposure to low temperature was different between varieties; Hayahikari showed a superior pod setting ability. In both varieties, compensative pod setting after the low temperature stress masked cold-weather damage. Hayahikari showed a greater capacity for pod setting during exposure to low temperature even in the long-term cooling treatment.

From these results, a high pod-setting ability during low temperature is a key factor in acquiring superior cold-weather tolerance. Hayahikari showed a great pod setting not only in the short-term but also in the long-term low temperature. This result demonstrates that Hayahikari showed a superior cold-weather tolerance in 1993, the most severe cold-weather damage in the last 50 years. Then, to add a Hayahikari-level pod setting ability to other breeding materials is a goal for the present in Hokkaido.

Although there is no doubt that an excellent pod setting under low temperature is the most stable trait for cold-weather damage, soybean breeders in Switzerland proposed that a compensative pod setting is one type of cold-weather tolerance (Schori et al., 1993). Flowers in a lateral raceme open several days after those in the central raceme at the same node in most of the soybeans. Therefore, soybean varieties whose flowers are sensitive to low temperature abscise most of the flowers in long-term low temperature like as Toyomusume. However, they found the interval between central and lateral flowering is about 10 days in some varieties and it is called asynchronous flowering (Schori and Gass, 1994). These varieties make up for a loss of abscised flowers due to low temperature in central racemes by pod setting in lateral racemes. The Tokachi Agricultural Experiment Station succeeded to introduce asynchronous flowering into breeding materials in Hokkaido by an indirect selection. These lines showed superior cold-weather tolerance in the long-term cooling treatment (Ohnishi et al., 2004). The results indicate that an asynchronous flowering is an effective against cold weather tolerance and is a practicable trait in Hokkaido, too.

Some papers (Narikawa et al., 1970; Hume and Jackson, 1981; Takahashi and Asanuma, 1996; Hayashi et al., 1998) evaluated cold-weather tolerance or pod setting ability in the cooling treatments for two weeks. However, as shown in this thesis, even in cold-weather sensitive varieties, damage by low temperature for the

short-term can be masked by compensatory pod setting after low temperature. Therefore, it is possible that an evaluation of cold-weather tolerance of soybeans by short-term cooling treatment makes a possibility of misinterpreting. Removing buds just after the treatment for excluding a compensative pod setting might be effective when short-term cooling treatment is used. But this work requires a great deal of time, and is not suited to test a lot of breeding materials. It can be concluded that long-term (four weeks) exposure to low temperature is appropriate to evaluate cold-weather tolerance of soybean varieties and breeding materials.

The effects of low temperature on pollination and fertilization in Hayahikari and Toyomusume were investigated (Chapter 4). Pollination was affected by cooling treatment in both varieties, but Hayahikari was significantly less damaged.

Hayahikari showed a significantly higher percentage of fertilized flowers than Toyomusume. A superior fertilization ability at low temperature is one of the factors required for cold-weather tolerance.

It was cleared that an excellent pod setting ability, shown in Chapter 3, is related to an ability of pollination. The result implies that soybeans with a superior ability of pod setting at low temperature can be selected from cross combinations by pollen number on stigma. However, to count numbers of pollen needs a lot of work, long-term cooling treatment is more practical to evaluate the cold-weather tolerance of soybeans in most cases. In the case of evaluation of soybean genetic resources which can not mature in Hokkaido, counting pollen numbers is more efficient than conducting a cooling treatment. It was reported that excellent cold-weather tolerant genetic resources against low temperature during flowering stage were found in tropical regions such as Indonesia, Malaysia and Laos in rice plants (Satake and Toriyama, 1979; Satake, 1981), and a variety and some parental lines were developed using the resources (Saito, 1988; Abe et al., 1989). It is possible that new cold-weather tolerant genetic resources would be found in warm regions in soybean, too. Soybean is a short day plant, and the growth naturally responds to day length, but the growth also responds to temperature. Many of the soybean germplasms collected from tropical regions can not often mature due to a shortage of accumulated temperature during the growth period in Hokkaido. For this reason, it is hard to evaluate their cold-weather tolerance rightly by pod number and seed yield in a cooling treatment. To grow soybeans until flowering period is enough to evaluate their cold-weather tolerance by pollen number on stigma under low temperature. This is very effective in evaluating the tolerance of soybean germplasm from warm regions. Thus, the Tokachi Agricultural Experimental Station is evaluating cold-weather tolerance of soybeans from East Asia by number of pollen grains on stigma at present.

In Chapter 5, the correlation of cold-weather tolerance with pubescence color and flowering time using near-isogenic lines (NILs) was investigated. The seed yield in tawny lines was significantly higher than that in gray lines in cooler regions. The dry matter production at the flowering stage was also higher in tawny lines than in gray lines in cooler regions.

The effect of cooling treatment on the pod number and seed yield of the NILs for a combination of pubescence color (T/t) and flowering time ($E1/e1$), te , tE , Te and TE , was examined. The cooling treatment slightly reduced the pod number and seed yield in the tawny lines (Te , TE) and in the late-flowering gray line (tE), but greatly reduced them in the early flowering gray line (te). Then, gray pubescence lines were grouped into early, mid and late flowering groups from two crosses. As a result of the cooling treatment, cold-weather tolerance was lowest in the earlier flowering group and cold-weather tolerance was highest in the later flowering group. These results show that selecting yellow-hilum soybeans with tawny pubescence and with

later flowering time are both useful for developing yellow-hilum soybeans with cold-weather tolerance.

In Chapter 5, it was suggested that two selecting methods seem promising to breed cold-weather tolerant soybeans with yellow hilum. One is to select tawny pubescence with yellow hilum soybeans, because the tawny pubescence gene *T* is related to the tolerance of cold-weather damage. To select tawny pubescence with yellow hilum soybeans (*TT/II*) is a simple procedure. Moreover, to use the gene *T* is an advantage regarding as suppression of seed coat pigmentation. Some of gray pubescence with yellow hilum soybeans (*tt/II*) develop a brown pigmentation around the hilum region in the seed coat due to low temperature in the flowering stage (Sunada and Ito, 1982; Ancha and Arihara, 1994). Soybeans with pigmentation are traded at low price. So far, the gene *T* (Takahashi and Asanuma 1996) and some of the maturity genes (Takahashi and Abe, 1999) were reported as the genes which suppress pigmentation. For this reason, when breeding tawny pubescence with yellow hilum soybeans (*TT/II*), it can be omitted that selection procedure of tolerance to a brown pigmentation around the hilum region in the seed coat. However, the combination of tawny pubescence and yellow hilum (*TT/II*) sometimes makes the colors of seed coat and hilum a dull gray. The seed coat color in tawny pubescence with yellow hilum soybeans (*TT/II*) is affected by genetic background or environmental conditions (Kurosaki and Yumoto, 2001). Furthermore, Cober et al. (1998) proposed the color classification 'imperfect yellow' to describe hilum color in tawny-pubescent yellow-hilum soybeans (*TT/II/rr*), and they also reported that this hilum color is affected by genetic background or environmental conditions, too. In the case of soybeans with a heavy dull gray color of seed coat and hilum, they would be poorly evaluated by soybean wholesalers. Thus, to breed soybeans with a bright seed coats and hilum is indispensable. Although genetic factors which affect the colors of seed coats and hilum in tawny pubescence with yellow hilum soybeans (*TT/II*) are unknown, some breeding lines which are tawny pubescence with fine colors of seed coats and yellow hilum have been successively selected in the Hokkaido Agricultural Experiment Stations. These lines showed superior cold-weather tolerance both in the cooling treatments and in cool regions as expected, and were highly evaluated as equal to other gray pubescence with yellow hilum soybeans (*tt/II*) by food processing companies.

Another method is to select soybeans with later flowering. It was shown that cold weather tolerance of *E1* lines was higher than that of *e1* lines both in tawny and gray pubescence isogenic lines, and later flowering soybeans have greater tolerance to damage due to low temperature at the flowering stage than earlier flowering soybeans in gray pubescent soybeans. Actually, to select soybeans with later flowering time is not complicated. The heritability of both flowering time and maturity is high in soybeans (Anand and Torrie, 1963; Kwon and Torrie, 1964), therefore to conduct the selection from the early generation is effective. However, late flowering soybeans with late maturity cannot accomplish whole growth in the northern and eastern parts of Hokkaido because the growth period is relatively short. Therefore, selecting soybeans which have late flowering and maturity should be avoided, and breeding soybeans which have later flowering time but not late maturity time is essential. It is usually observed that variance in the flowering time among soybeans with similar maturity time in genetic resources. Nelson (1988) conducted selections in terms of the flowering time while maintaining similar dates of maturity, and soybean lines with similar time in maturity differed by as much as almost one month in the flowering time. Actual selection can be carried out by marking soybeans with late flowering and then with the date of maturity time. The flowering time of group E is classified in 'early' (according to the report of Japanese soybean characteristics classification 1995: in the cold

latitudes); that of Group M is 'medium early' and that of group L is from 'medium' to 'medium late' in the experiment. In the Tokachi Agricultural Experiment Station, soybean lines whose flowering time is from 'medium' to 'medium late' and maturity time is 'early medium' (which is a practical maturity time in eastern Hokkaido), were selected using the way mentioned above. These lines showed a good cold-weather tolerance in the cooling treatment. Consequently, when taking practical maturity time in Hokkaido into consideration, the optimal flowering time to select for a cold-weather tolerant soybean would be from 'medium' to 'medium late'.

From what has been shown above, it is concluded that an excellent pod-setting ability under low temperature and less sunshine, a superior fertilization ability at low temperature, tawny pubescence with superior growth vigor and later flowering are important factors to improve cold-weather tolerance.

Finally, I describe problems to be solved with regard to researches on cold-weather tolerance in the future. In this thesis, it was shown that an excellent pod-setting ability, a superior fertilization ability, tawny pubescence and later flowering are effective factors to improve cold-weather tolerance. Compensative pod setting proposed by Schori et al. (1993) is also promising one. However, there is still a possibility that each trait is not sufficient in very severe cold summer. In the first place, it is necessary to introduce these traits into other breeding materials. Besides, to improving the cold-weather tolerance further, it is important to develop a variety with the compound traits.

Usually, there are several breeding objectives in a breeding program. It is difficult to unite a high level of cold-weather tolerance with other important characteristics such as high yielding, disease resistances and food processing suitability. In order to overcome the difficulties, to clarify physiological mechanism of cold-weather tolerance from different points of view, mode of detail inheritance or connections with other agronomic traits would give us helpful hints. These findings lead to develop other indirect selections or marker-assisted selections.

Mother plants are one of the most important factors in breeding strategy. So far, the Tokachi Agricultural Experiment Station has been used as indigenous germplasm which originates from Sakhalin, eastern Hokkaido and Sweden as mother plants of cold-weather tolerance. It is not very long since soybean breeding for cold-weather damage was started in the world. Thus, few publications are available with regard to cold-weather tolerant varieties. However, some cold-weather tolerant varieties have been developed in North America and Europe which are at high altitude for a little over these ten years. The Tokachi Agricultural Experiment Station introduced genetic resources related to cold-weather tolerance from Europe and North America (Yumoto, 1994; Kurosaki, 1998). Some of them have been used as mother plants. It is very important to introduce new genetic resources including their origin even in warm region and to evaluate their cold weather tolerance continuously to find an excellent mother plant.

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*In Japanese with English summary.

** In Japanese (Some of the titles are tentatively translated).

Summary

Hokkaido, which is the largest crop production area in Japan, is located in northern Japan. Cool summers often do the damage to soybeans as well as rice plants in Hokkaido. However, studies related to cold weather-damage and the tolerance in soybeans are much fewer than those in rice plants in our country. Poor growth due to low temperature in the beginning of growth, abscission of flowers and pods due to low temperature during the flowering stage and insufficient grain filling due to low temperature in the pod-filling stage are the three major factors reducing soybean yields affected by cold weather. The most common damage by low temperature is poor growth, but abscission of flowers and pods causes the most severe damage in soybean yield. There are few effective cultivating measures against cold- weather damage, and breeding is the best countermeasure in soybean. Although detail studies have done on breeding for cold-weather damage due to poor growth, very few researches which play a part in breeding for the damage due to flower and pod abscission have been made. In 1993, very severe low temperature during flowering season ruined soybean production in Hokkaido. Thereafter, it has been recognized that improving cold-weather tolerance during the flowering stage is dispensable to stabilize soybean cultivation. Accordingly, it is very important to conduct researches which contribute to soybean breeding for cold-weather damage caused by flower and pod abscission.

In this thesis, the author analyzed the soybean growth focusing on environmental factors in regard to cold-weather damage. Response to pod setting under low temperature and mechanisms of the tolerance in varieties with different levels of cold-weather tolerance were clarified. The relations between the cold-weather tolerance and agronomic characteristics were investigated in order to develop a cold-weather tolerant variety by an indirect selection. The summary of the results which obtained in the experiments are as follows.

The effects of low temperature and shading during the flowering season on soybean growth were analyzed in two varieties of soybeans: Hayahikari, an excellent cold-weather tolerant variety, and Toyomusume, a cold-weather sensitive variety. Hayahikari showed the lowest seed yield reduction among the soybean varieties and lines in 1993; very severe damaged year by low temperature. The soybean plants were exposed to low temperature of 18 °C day / 13 °C night, shaded (50%) without cooling treatment, or shaded at low temperature, during the four-week flowering season. Effects of cooling and shading treatments on the vegetative growth were small in either variety. The reproductive growth was affected by low temperature and shading in both varieties. Low temperature during the flowering season markedly reduced the seed yield, cold-weather damage is mainly caused by low temperature. A varietal difference in the seed yield after the cooling treatment is attributed to the difference in the response of all the yield components to the low temperature, especially the number of pods per plant,. However, both varieties showed the similar susceptibility to shading. The results indicate that to breed a variety which has a superior pod setting ability not only at low temperature but also under insufficient sunlight would be an effective way to improve cold-weather tolerance (Chapter 2).

The effects of short-term exposure (two weeks) to low temperature and long-term exposure (four weeks)

from the beginning of flowering on pod setting were examined in Hayahikari and Toyomusume. The short-term exposure to low temperature did not decrease the total number of pods even in Toyomusume. However, pod-setting ability during low temperature exposure was different between varieties; Hayahikari showed a superior pod setting ability to Toyomusume. After the short-term exposure to low temperature, pod setting showed recovery from the cold-weather damage by compensative pod setting after the cooling treatment in both varieties. The total number of pods in the long-term cooling treatment was remarkably lower in Toyomusume than in Hayahikari. Toyomusume sustained damage not only during low temperature but also after it. On the other hand, Hayahikari showed a greater capacity for pod setting during low temperature stress (Chapter 3).

The effect of low temperature on pollen activity, pollination and fertilization in Hayahikari and Toyomusume were investigated. Pollen germination rates were lowered in Toyomusume by cooling treatment for just four days, a sensitivity of pollen activity to low temperature was high. Pollination was affected by the cooling treatment in both varieties, but Hayahikari was significantly less damaged. Hayahikari showed a significantly higher percentage of fertilized flowers under low temperature than Toyomusume. Since a close relationship was observed between the pollen number per stigma and the percentage of fertilized flowers, it can be concluded that the effect of low temperature on pollination is closely related to the fertilized flowers. A superior fertilization ability at low temperature is one of the factors required in cold-weather tolerance (Chapter 4).

Brown-hilum soybeans often have cold-weather tolerance and growth vigor superior to that of yellow hilum soybeans. However, Japanese consumers do not prefer brown-hilum soybeans, therefore, to improve cold-weather tolerance of yellow hilum soybeans has been a major objective in Hokkaido. The correlation of cold-weather tolerance with pubescence color and flowering time, which are supposed to be related to the tolerance, using near-isogenic lines (NILs) were investigated. First, the NILs for pubescence color (tawny: *T* and gray: *t*) of yellow-hilum soybeans were grown under various temperature conditions, and their growth and seed yield were examined. There was no significant difference in the seed yield between the tawny and gray lines in a warm region, but the seed yield in tawny lines was significantly higher than that in gray lines in cooler regions. The dry matter production at the flowering stage was also higher in tawny lines than in gray lines in cooler regions (Chapter 5).

Next, the effect of cooling treatment on the pod number and seed yield of the NILs for a combination of pubescence color (*T/t*) and flowering time (*E1/e1*), *te*, *tE*, *Te* and *TE*, was examined. The cooling treatment from the beginning of flowering slightly reduced the pod number and seed yield in the tawny lines (*Te*, *TE*) and in the late-flowering gray line (*tE*), but greatly reduced them in the early flowering gray line (*te*). Then, gray pubescence lines were grouped into early, mid and late flowering groups from two crosses, and they were exposed to low temperature. The rates of reduction in the pod number and seed yield by the cooling treatment were the highest (cold-weather tolerance was the lowest) in the earlier flowering group and the lowest (cold-weather tolerance was the highest) in the later flowering group. Judging from the magnitude of the effects of maturity genes which have been reported so far, it is possible to think that earliest flowering group is

el/el and latest group is *E1/E1* in the experiment, there is a possibility that the *E1* allele or closely linked gene is related to cold-weather tolerance. These results show that selecting yellow-hilum soybeans with tawny pubescence and with later flowering time are both useful for developing yellow-hilum soybeans with cold-weather tolerance (Chapter 5).

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北海道におけるダイズ開花期の耐冷性の支配要因の解析とその育種への応用

黒崎英樹

要約

北海道は、日本最大の作物生産地帯である。北海道のダイズはイネ同様に頻繁に冷害に遭遇しているものの、わが国におけるダイズの冷害および耐冷性に関する研究は、イネに比べて格段に少ない。低温による減収の要因として、①生育初期の低温による生育不良、②開花期の低温による落花、落莢および③登熟期の低温による子実の肥大不良の3つが挙げられる。冷害の中で頻度が最も高い減収要因は、生育不良型冷害であるが、開花期の低温に起因する障害型冷害による減収が最も著しい。ダイズにおいては、冷害に対する有効な耕種的対策は少なく、耐冷性品種を栽植することが一番有効な対策である。頻度の高い生育不良型冷害に対する育種に関する研究は数多く行われてきたが、障害型冷害に関しての育種的研究は不十分であった。1993年の著しい障害型冷害により、北海道のダイズ生産は壊滅的被害を受けたことから、開花期の低温抵抗性向上が北海道におけるダイズの安定生産には不可欠であり、開花期低温抵抗性育種に寄与する研究の重要性が認識された。

本論文で、筆者は、ダイズの生育を冷害に関係する環境要因から解析するとともに、低温抵抗性の異なる品種を用いて、低温下での着莢反応および低温抵抗性の機作について明らかにした。また、間接選抜による低温抵抗性品種の育成に資するため、低温抵抗性と農業形質の関係を調査した。一連の実験から得られた結果の要約は、以下の通りである。

(1) 開花期の低温と遮光がダイズの生育に及ぼす影響

1993年の冷害では、生育期間全般が低温に推移したが、特にダイズの開花期に相当する7月下旬から8月上旬の平均気温が、平年より約6℃低い15℃であり、また、日照時間も少なく、7月下旬

の日照時間は、わずか1.4時間であった。この厳しい低温と少照が着莢障害に関与しているものと考えられたことから、低温と遮光がダイズの生育に及ぼす影響を調査するため次の試験を行った。

耐冷性強のダイズ品種「ハヤヒカリ」と耐冷性中の「トヨムスメ」を用いた。「ハヤヒカリ」は1993年の著しい障害型冷害年において、当時の既存品種が大きく減少する中であって、育成材料中最も被害が少なかった系統（当時「十系802号」）である。その後、本論文の試験を含め耐冷性を確認し、また、その他の各種試験を経て、1998年に「ハヤヒカリ」として品種登録されている。

試験は、平常年の1997年に1/2000aのポットを用いて、対照区は全期間屋外で栽培し、処理として低温処理（昼18℃/夜13℃）、常温での遮光処理（50%）および低温＋遮光処理の3区を設け、開花期に4週間にわたって処理を行った。低温処理の18℃は複数の障害型冷害年の開花期における平均気温、13℃は1993年の最低平均気温により設定した。

低温と遮光が栄養生長に及ぼす影響は小さく、低温処理、遮光処理、低温＋遮光処理区の栄養生長に関する形質（主茎節数、分枝数、総節数）は対照区並であった。一方、生殖生長は両品種ともに低温と遮光の影響を受けた。開花期の低温処理により子実重は低下し、品種間差が見られ（「トヨムスメ」対照区対比13%、「ハヤヒカリ」同77%）、開花期の冷害は主に低温による被害が大きいことが示された。

低温による子実重の品種間差は、全ての収量構成要素（莢数、一莢内粒数、百粒重）の差に起因していた。すなわち、「トヨムスメ」は収量構成要素全てが低温により有意に減少したが、「ハヤヒカリ」は一莢内粒数のみが有意に減少した。減

少程度は「トヨムスメ」が「ハヤヒカリ」をいずれの形質も上回った。低温により最も品種間差が現れたのは莢数（稔実莢数を示す 以下同じ）であった（「トヨムスメ」対照区対比 36%、「ハヤヒカリ」同 94%）。

遮光の影響は莢数に現れたが、他の形質への影響は少なく、処理により莢数が両品種共に有意に減少し、その減少程度は同程度であった（「トヨムスメ」対照区対比 82%、「ハヤヒカリ」同 80%）。

低温＋遮光処理による影響は、多くの形質では、低温処理単独のものと大差なかったが、莢数に及ぼす影響は大きく、低温処理と遮光処理が複合した影響となった。低温処理に比べて莢数が有意に減少し、この影響は両品種ともに見られた。「トヨムスメ」は、低温処理区対照区対比 36%が低温＋遮光区は同 28%に、「ハヤヒカリ」は低温処理区同 94%が同 81%に、それぞれ減少程度が大きくなった。

葉温が光合成や転流に関与していると報告されていることから（Marowitch ら 1986）、低温区および低温＋遮光区の葉温を、晴天と曇天時に測定した。低温区および低温＋遮光区の葉温は、曇天下では、両区共に気温とほぼ同じであった。しかし、晴天時には、低温区の葉温は気温に比べてかなり高かったが、低温＋遮光区の葉温は気温とほぼ同じであった。このため、低温に遮光が加わると、葉温が低下し、生理的機能が低下するため、低温区に比べて、子実重と莢数の減少程度が大きくなった理由の一つと考えられた。

これら結果から、他の形質に優れる耐冷性中レベルのダイズの耐冷性向上には、収量構成要素全ての改良が必要であること、特に重要なのは低温に対する着莢能力であることが示された。さらに寡照下での着莢能力を向上させることが、現在の低温抵抗性レベルを向上するために必要であることが示唆された。

(2) 開花期の低温に対する着莢反応の解析

開花期の低温に対する品種間差に最も寄与するのは莢数であることから、低温が着莢に及ぼす知見を得るために本試験を行った。

前章と同じく「ハヤヒカリ」と「トヨムスメ」をポット栽培し、対照区は開花始から 4 週間、昼 23℃/夜 18℃で生育させ、低温処理として 2 処理を行った。低温処理は、開花始から短期の低温[18℃/13℃ (昼/夜) 2 週間]と長期の低温[18℃/13℃ (昼/夜) 4 週間]である。低温処理には遮光処理 (50%) を併せて用いた。調査は開花 1 週間毎の着莢数、着莢率について行った。

低温処理により花数は増加し、開花期間は延長し、着莢はそれに伴い遅延する傾向が見られた。

短期の低温処理では、両品種共に莢数は、対照区に比べて減少しなかった（「トヨムスメ」対照区対比 99%、「ハヤヒカリ」同 108%）。しかし、着莢並びに着莢率の推移には品種間差が見られ、「ハヤヒカリ」は低温処理中の莢数は対照区と比べて減少したものの、その程度は小さく、かつ着莢率が対照区とほぼ同様に推移したのに対して、「トヨムスメ」は低温処理中の着莢数の低下程度が「ハヤヒカリ」より大きく、着莢率も低下した。短期の低温処理によっても処理中の着莢率に影響を及ぼし、品種間差が認められた。

両品種共に短期の低温処理における莢数は対照区並みであったが、低温処理中の着莢の割合は、「トヨムスメ」が全体の約 3 割、「ハヤヒカリ」は 6 割であった。すなわち、「トヨムスメ」は低温処理後に開花した花による着莢が主であった。短期の低温処理であれば、処理中の着莢数が減少した場合、処理後の着莢により補うことがわかった。

長期の低温処理では、「トヨムスメ」は低温処理中に加えて、処理後も着莢率が低く、短期の低温処理で見られた処理後の着莢が少なかったため、大きく着莢数が減少した。「ハヤヒカリ」の着莢率の推移は、短期の低温処理とほぼ同等で総莢数の減少程度は「トヨムスメ」より極めて小さく、低温処理中に全ての着莢が行われたと共に低温処理中の着莢能力が高かった。また、低温下での着莢率の品種間差は、開花前半 2 週間が特に大きかった。長期処理における開花前半 2 週間の花は、短期処理における同時期の花に比べて、開花後 2

週間定温に曝されていることになり、開花後の低温に対して品種間差が存在することが示された。

いくつかの論文では、ダイズの耐冷性を2週間（短期）の低温処理で評価しているが、低温に感受性の品種でも低温後の着莢によりその障害がマスクされ、解釈を誤る恐れがあるので、4週間程度の長期の低温処理により耐冷性を評価すべきである。

(3) 開花期低温抵抗性機作の解明

前章において、短期の低温処理でも処理期間中の着莢率に品種間差が認められた。すなわち、「ハヤヒカリ」の着莢率が、「トヨムスメ」より高かった。一方、ダイズの雄蕊は雌蕊に比べて、低温に対する感受性が高いと報告されている（後藤・山本 1972）。そのため、低温の影響が最初に雄蕊に現れると考えられることから、低温が受精に及ぼす影響を雄蕊について調査した。

①低温が花粉発芽率に及ぼす影響

ポット栽培した「トヨムスメ」を開花始から11日間、昼15℃/夜10℃の低温処理を行い、2日毎に葯を採取し、25℃に保持した恒温器の寒天培地に6時間静置し、花粉発芽率を計測した。

花粉発芽率は、低温の影響を受け低下し、低温処理11日目の花粉発芽率は約20%であった。また、「トヨムスメ」の花粉発芽能力は、わずか4日間の低温処理により低下し、花粉の活性が低温に敏感であることが伺えた。

②低温が受粉に及ぼす影響

ポット栽培した「ハヤヒカリ」および「トヨムスメ」を開花始から1週間、昼18℃/夜13℃の低温処理を行い、2日毎に受粉数として柱頭上の花粉数を計測した。ダイズの受精に必要な花粉数はわかっていないが、ダイズより数倍大きい柱頭を持つアズキでは、柱頭上に100以上受粉すると正常に受精すると報告されていることから（島田 1990）、この知見に基づき、101以上の受粉数を正常受粉数と見なして評価した。101以上の受粉数は「ハヤヒカリ」、「トヨムスメ」ともに低温処理日数の経過に伴い低下したが、「トヨムスメ」の低下割合は「ハヤヒカリ」より大きく、7日目における受粉数101以上の花の比率は、「トヨム

スメ」は22%、「ハヤヒカリ」は46%であり、品種間に有意差が認められた。

③低温が受精に及ぼす影響

ポット栽培した「ハヤヒカリ」および「トヨムスメ」を開花始から2週間、昼18℃/夜13℃の低温処理を行い、開花始から2日毎に1週間の受精花率を調査した。なお、調査は低温処理終了後に行った。

受精花率の推移は先の実験における101以上の受粉数と同様の傾向を示した。低温処理の経過に伴い、両品種共に低下したが、低下程度には品種間差が見られ、「ハヤヒカリ」に比べて「トヨムスメ」は急激に低下した。7日目の受精花率は、「トヨムスメ」は11%、「ハヤヒカリ」は68%であり、この受精花率の推移は品種間で有意差が認められた。

受粉数の多少に関する因子の一つとして葯当たり花粉数が挙げられ、イネでは葯当たり花粉数と葯長に高い正の相関があること、通常温度条件下でも耐冷性品種は葯が長いことが報告されている（鈴木 1981、1982）。「トヨムスメ」と「ハヤヒカリ」の葯長を調査した結果、両品種間に差が見られなかったことから、葯当たり花粉数は、ダイズにおいて耐冷性の品種間差の要因ではないと考えられた。

以上から、柱頭上の花粉数と受精花率との間に密接な関係が認められたことから、低温下の受粉数が受精と深く関わっていると結論された。また、低温下の優れた受精能力が、開花期の低温抵抗性獲得には不可欠な要素であると考えられた。

柱頭上の花粉数の計測は、労力を要するため、低温処理による莢数や子実重の減少程度に基づいて耐冷性を評価する方が实际的である。しかしながら、柱頭上の花粉数の計測による耐冷性の評価は、開花期まで栽培することで耐冷性の評価を行うことができる。イネでは熱帯地方由来の遺伝資源から耐冷性の母材が見つかった（Satake and Toriyama 1979、佐竹 1981）。ダイズにおいてもその可能性はあり、柱頭上の花粉数の計測による耐冷性の評価は、北海道で容易に成熟しないダイズの耐冷性の評価に有効であると考えられる。

(4) 耐冷性白目ダイズ育成を目的とした農業形質と耐冷性との関係解析

一般的に、種皮色が黄色のダイズの臍色は大きく褐目と白目に分けられる。褐目ダイズは、耐冷性や生育量が白目ダイズより優っているが、日本の消費者は、見栄えが劣る理由から褐目ダイズを好まないため、白目ダイズの耐冷性向上が北海道における主要な育種目標となっている。一方、現在の耐冷性の選抜は、冷涼地圃場や人工気象室に供試し、その反応に基づく直接選抜により実施している。しかしながら、圃場選抜は、高温年には十分な選抜ができないことや人工気象室を用いた選抜は、施設の面積により供試できる材料数に制限がある等の問題がある。間接的な選抜が可能となれば、早期世代から、大量の育種素材をスクリーニングすることができる。ダイズ育種上の観察から次の2形質に着眼した。

北海道のほぼ全ての褐目品種 ($i-i/i-i$) の毛茸は褐毛 (l)、白目品種の毛茸は白毛 (t) であるが、褐目・褐毛ダイズと白目・白毛ダイズの交雑後代が、臍色にかかわらず、褐毛ダイズの生育量が優ることを観察した。また、同一組合せの交雑後代のうち、開花期の早い系統より開花期が遅い系統の生育量が優ることも観察している。低温下での生育量と耐冷性に関係があるとの報告 (三分一 1979) がなされていることから、毛茸色並びに開花期が耐冷性に関与していると想定し、これら2形質と耐冷性との連関について、同質遺伝子系統を用いて調査するとともに、選抜実験を行った。

①毛茸色に関する白目の準同質遺伝子系統の冷涼地における生産力および低温下での光合成能力

毛茸色に関する白目の準同質遺伝子系統 (褐毛: T 白毛: t) を温度環境の異なる圃場で生育させ、生育量と子実重を調査した。

供試箇所は標準的な気象の芽室と冷涼地3箇所 (上士幌、大樹、訓子府) の計4箇所である。供試材料は、十勝農試で養成した毛茸色に関する準同質遺伝子系統2組合せ4系統、栽培方法は北海道の標準栽植密度 (約18000本/10a) および施肥

量 ($N: 2-P_2O_5: 18-K_2O: 9\text{kg}/10\text{a}$)、乱塊法4反復で行った。

莢数および子実重は、生育期間中の平均気温が低いほど少なくなる傾向であった。芽室での子実重は、褐毛系統と白毛系統間に有意差は見られなかったが、冷涼地3箇所での子実重は、褐毛系統が白毛系統より有意に高かった。また、開花期における生育量は子実重と同様の傾向を示し、葉、茎、根および根粒の乾物重は、芽室では白毛と褐毛系統間に差はみられなかったが、冷涼地においては褐毛系統が白毛系統より高かった。

また、上記に供試した毛茸色に関する準同質遺伝子系統のうち1組合せ2系統をポット栽培し、開花期に人工気象室で温度を変えて光合成速度を調査した。その結果、光合成速度は、温度が低いほど低下し、全般に褐毛系統が白毛系統を上回っていた。しかし、褐毛系統が白毛系統を上回る程度は、標準 (昼23℃/夜18℃) の温度条件に比べて、低温下 (昼18℃/夜13℃、昼15℃/夜10℃) において、その割合が大きかった。

これらの結果から、褐毛・白目の形質にすることにより、耐冷性が向上し、その耐冷性には、低温下での生育量の旺盛さと光合成能力が関与しているものと考えられた。

褐毛・白目 (TT/II) の遺伝子型の組合せは種皮や臍にくすみを生ずることがあり、くすみの程度には遺伝的要因または環境要因が関与していると報告されている (Cober ら 1998、黒崎・湯本 2001)。くすみの程度に関与する遺伝的要因は明らかになっていないが、十勝農試においてくすみがほとんど問題とならない褐毛・白目ダイズが既に育成され、これらダイズの蒸煮後の種皮色は白毛・白目ダイズとほぼ同等である。

②毛茸・開花期と耐冷性との関係解析

まず、毛茸色 (T/t) と開花期 (EI/eI) の組合せの準同質遺伝子4系統 (te 、 tE 、 Te 、 TE) を用いて、低温処理が莢数と子実重に及ぼす影響を調査した。 EI は開花に関する遺伝子のうち最も作用力の大きい遺伝子で、 te (白毛・開花期早)、 tE (白毛・開花期晩)、 Te (褐毛・開花期早)、 TE (褐毛・開花期晩) である。これら系統を開花始から4週

間低温処理（昼 18℃/夜 13℃）を行った結果、褐毛の2系統(Te 、 Td)は、開花期の早晚にかかわらず、莢数と子実重の減少は少なく、褐毛が耐冷性に関与していることを支持する結果であった。一方、白毛系統は、開花期の早晚により傾向が異なり、開花期の早い te の莢数と子実重の減少程度は大きかったが、開花期の遅い白毛系統(td)の同形質の減少程度は褐毛系統並みであった。本結果を分散分析で解析したところ、低温処理に対して、褐毛内では系統間に有意差が認められなかったが、白毛内系統間では莢数、子実重共に有意差が認められ、白毛でも開花期の遅い系統を選抜することにより耐冷性が向上する可能性が示唆された。

次いで、2組合せの交雑後代の白毛系統を開花時期によって分類し（開花期：早、中、晩）、選抜した。 F_2 、 F_3 は無選抜、 F_4 、 F_5 において開花期によって選抜し、 F_6 で開花始から4週間の低温処理（昼 18℃/夜 13℃）を行った。その結果、両組合せ共に開花期の早いグループは莢数と子実重の減少程度が大きく、開花期の遅いグループほど減少程度が小さかった。開花期の遅いグループの耐冷性は、耐冷性強の標準品種と同程度であった。これまで報告されている熟期遺伝子の効果の大きさから見て、本試験における最も開花の早いグループは $e1/e1$ 、最も開花の遅いグループは $E1/E1$ と想定され、 $E1$ が耐冷性に関与している可能性が考えられた。

上記結果から、開花期が遅いことが耐冷性向上に有効であることが示された。ただし、生育期間の短い北海道において、開花期の遅い個体を選抜する場合に、成熟期も遅くすることは避けなくてはならない。登熟期間が短い形質との複合が求められる。同時期の成熟期で登熟期間に大きな変異をもたせる選抜が可能なが報告されていること（Nelson 1988）、十勝農試の遺伝資源においても、同時期の成熟期でも登熟期間には大きな変異が見られることを観察していることなどから、今後、開花までの期間と登熟期間の変動について整理することが必要である。