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REVIEW

Recent Advances in Physiological and Genetic Studies on Chilling Tolerance in Soybean

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Abstract

Low temperatures cause a decrease in seed yield and deterioration of seed quality in soybeans and have threatened stable soybean production in cool areas. To overcome this problem, physiological and genetic analyses have been conducted as steps towards the development of efficient breeding systems. Several evaluation methods that are useful for physiological studies as well as for genetic screening have been developed. Low temperature treatment for four weeks or longer starting at the first flowering stage in plants was found to reduce all of the yield components and result in genotypic variation in chilling tolerance for seed yield during reproductive growth. Analysis of low seed fertility induced by low temperature treatment prior to flowering revealed that the tetrad stage during pollen development is the most sensitive to chilling. Browning of seed coats, a major factor causing deterioration of seed quality, was induced most severely by low temperature treatment about one week after flowering. In addition, several genetic loci associated with chilling tolerance have been identified. Cultivars and lines with the *T* allele at the *T* locus, which controls the color of pubescence, have repeatedly been demonstrated to exhibit better chilling tolerance than those with the other allele, *t*. Maturity loci controlling flowering time and maturity such as *E1*, *e3* and *e4* were also found to be associated with chilling tolerance for both seed yield and quality. However, evidence showing that these loci are directly involved in chilling tolerance remains to be provided.

Discipline: Plant breeding
Additional key words: browning in seed coats, evaluation methods, genetic loci

Introduction

Many of the world's important crops are now widely distributed beyond their original zones of natural selection and hence their yields are constrained by what have been termed thermal thresholds for optimal growth[8]. The cultivation of soybean, which prefers a warm climate, has continued its expansion into cool areas, including Canada, Sweden, Siberia, and Hokkaido, the northernmost island of Japan, where low temperature is a major constraint for seed yield (Fig. 1). Low temperature during the period of reproductive growth also causes deterioration of seed quality by inducing browning and cracking in seed coats (Fig. 2A).

Since it is difficult to overcome chilling injury by modification of cultivation conditions, many breeding efforts have been made in cool areas. In the course of breeding chilling-tolerant cultivars, breeders and related researchers have developed methods to screen genetic resources and to evaluate the degrees of chilling tolerance of lines/cultivars as well as to elucidate the physiological and genetic mechanisms underlying chilling tolerance. Intensive studies have continuously been conducted at the Hokkaido Prefectural Tokachi Agricultural Experiment Station (TAES) in Hokkaido, Japan. Breeders at the station have made contributions not only by conducting physiological and genetic studies but also by providing

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attractive plant materials for fundamental research, which have activated this research area in Japan.

Here, we review recent advances in physiological and genetic studies on soybean chilling tolerance, focusing on those conducted in Japan.

Chilling tolerance for seed yield

1. Physiology and evaluation methods

Low temperatures in the field reduce every yield component (total pod number, seed number per pod, single seed weight) of soybean, resulting in drastic reduction in seed yield[21]. To reproduce this phenomenon, called cool injury or chilling injury, along with the genetic variations in chilling tolerance among cultivars under defined climatic conditions, many temperature regimes and evaluation methods for chilling tolerance have been tested since growth chamber facilities called phytotrons were established at TAES and Hokkaido Agricultural Experiment Station (present name: National Agricultural Research Center for Hokkaido Region). Chilling tolerance has been evaluated by monitoring how small the reduction of seed yield and yield components in the chilling environment is relative to those in the control environment. Therefore, the experiments have basically involved control plants, which are grown under conditions identical to those for chilled plants except for the temperature during low-temperature treatment. Although low-temperature (12–15°C) treatment was applied for approximately two weeks in early studies, a low level of genetic correlation between the growth chamber and the field conditions, which may be due to the relatively long flowering period and the compensation ability in soybean, has been pointed out to be problematic[20]. Kurosaki et al.[13] clearly showed that there was no genotypic difference in total number of pods even between cultivars typically differing in chilling tolerance after two weeks of treatment, although short-term exposure may be used to evaluate pod setting ability during the low-temperature treatment.

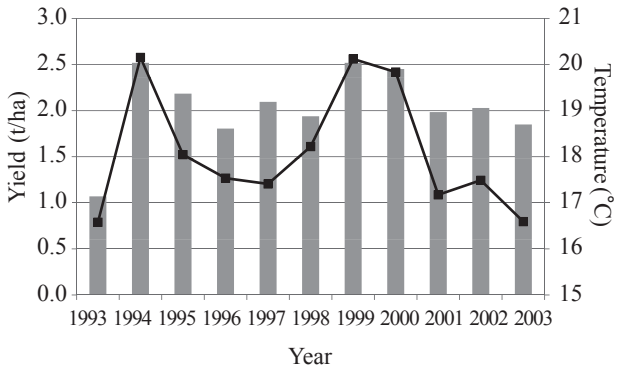

Fig. 1. Relationship between soybean yield and summer temperature

Bars indicate the mean yields in Hokkaido. Closed squares indicate the mean temperatures at Obihiro, Hokkaido from July to September. The values were calculated using Amedas data compiled by the Japan Meteorological Agency and data obtained by a survey by the Ministry of Agriculture, Forestry and Fisheries, Japan (http://www.maff.go.jp/soshiki/nousan/hatashin/daizu/siryo/index.html).
: Yield, —■— : Temperature.

Kurosaki and Yumoto[12] established a method for evaluating chilling tolerance with long-term exposure to a low temperature (four weeks at 18°C/13°C) starting at the time of flowering (Table 1, method A). This condition was proven to reproduce quite well the genetic differences seen in the field under the growth chamber condition[34,40]. Under this condition, all of the yield components in a sensitive cultivar, Toyomusume, decreased more se-

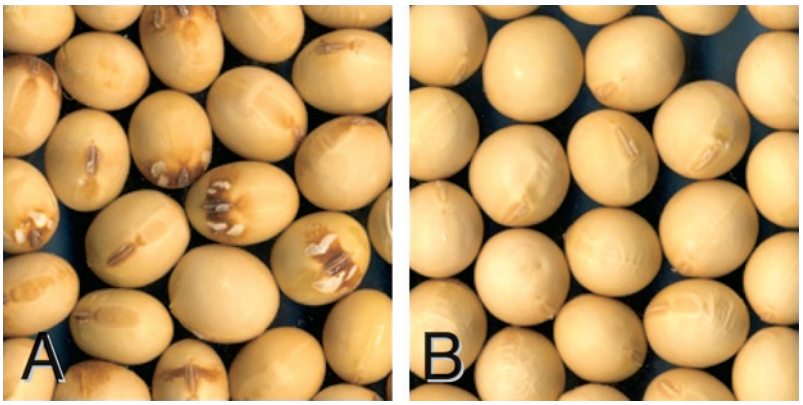

Fig. 2. Effect of low temperatures on appearance of soybeans

A: Seeds of a chilling-sensitive cultivar, Toyomusume. B: Seeds of a chilling-tolerant cultivar, Toyoharuka.

verely than did those in a tolerant cultivar, Hayahikari[12]. Among the yield components, the greatest genotypic difference was found in the decrease in total pod number. A responsible factor was determined to be the lower fertilization percentage in Toyomusume[13], although this did not explain all of the difference. Shading, which simulated typical cloudy weather in cool summer in Hokkaido, enhanced the chilling injury, regardless of the cultivar[12].

Although method A is very useful for evaluating lines in the breeding program for the development of cultivars adapted to Hokkaido, a few modified methods have been devised to improve reproducibility and versatility[5]. In these methods, plants including control ones were grown under controlled temperature regimes for their entire lifetimes (Table 1). While method B in Table 1 resembles method A, method C can reveal latent chilling tolerance in plants with a wide variation in flowering time and maturity, since the treatment began at the same developmental stage of first flowering and was completed at maturity. Despite the long-term low-temperature exposure, genetic correlation in chilling tolerance between the field and this condition was observed[5]. Although natural light, which is variable, was used as the light source in methods B and C, it was shown that the genotypic difference in chilling tolerance was maintained, regardless of the growing season (spring-summer or summer-autumn), which is consistent with the results obtained in the shading experiment described above.

Very recently, Ohnishi and Shirai[17] proposed a method for determining fertilization ability using low-temperature treatment for plants before anthesis (Table 1, method D). In rice, the major factor reducing seed yield at low temperatures is known to be the low seed fertility resulting from decrease in the number of normal fertile pollens. The most sensitive stage was found to be the booting stage or, more precisely, the beginning of the microspore stage[22]. Fertilization of soybean flowers has also been examined in some studies. However, precise monitoring of pollen fertility at low temperatures seems to have been hindered by several characteristics of soybean such as little trace of flowers after shedding, natural dropping of flowers even under favorable conditions and pod shedding in the course of maturation. To realize uniform stages of flowers and maximum fertilization percentage estimated by the ratio of the mature pod number to the flower number in a control environment, Hashimoto & Yamamoto[9] developed a method in which only a few flowers on a few nodes were left for analysis with the others being removed. Although their studies revealed that nitrogen nutrition influences fertilization efficiency at a low temperature, their method is not cur-

Table 1. Growth conditions in evaluation methods for chilling tolerance in soybean

Method	Treatment	Conditions before treatment			Time for starting treament	Conditions during treatment			Period of treatment	Condition after treatment			Ref.
		Environ.	Temp.	Light		Environ.	Temp.	Light		Environ.	Temp.	Light	
A	Control	Outside	Ambient	Ambient	First flowering	Outside	Ambient	Ambient	4 weeks	Outside	Ambient	Ambient	12
	Chilling					GC	18°C/13°C						
B	Control	GC[1)]	22°C/17°C	Lamp/15h[2)]	First flowering	GC	24°C/17°C	Ambient	4 weeks	GC	20°C/16°C	Ambient	5
	Chilling						15°C/15°C	+lamp/15h[3)]				+lamp/15h	
C	Control	GC	22°C/17°C	Lamp/15h	First flowering	GC	24°C/17°C	Ambient	Until maturity	–	–	–	5
	Chilling						15°C/15°C	+lamp/15h			–		
D	Control	GC	25°C/20°C	Ambient	14–17d before anthesis[4)]	GC	25°C/20°C	Ambient	1 week	GC	25°C/20°C	Ambient	17
	Chilling						15°C/10°C						

1): Growth chamber.
2): Metal halide lamp for 15h light.
3): Ambient light with supplementary lamp for 15h light.
4): Buds at the corresponding stage will be examined.

rently used, probably due to the small number of flowers for estimation and the unusual physiological state of plants. Ohnishi and Shirai[17] proposed a method for accurate estimation of fertilization percentage by the use of a new index; they monitored the percentage of elongated pods per flowers several days after anthesis, instead of the conventional index of pod set percentage at maturity. By using this method, they determined that the most sensitive stage during pollen development is around the tetrad stage. It is interesting that the degree of chilling tolerance determined by method D was different from that determined by method A in some cultivars, suggesting the existence of different genetic factors responsible for chilling tolerance at the two developmental stages and the possibility of pyramiding the chilling tolerance genes for higher stability of the yield level.

2. Genetic loci associated with chilling tolerance

Soybean breeders and researchers have identified several genes associated with chilling tolerance. They started by searching for genes that are commonly present in chilling-tolerant cultivars, based on the correlation between the traits controlled by known genetic loci and the response of genotypes to variable temperatures in different locations and years. Using this approach, several loci such as *P1/p1* (glabrous/pubescent surface)[21] and *APX1* (ascorbate peroxidase 1)[4] have been identified as possible loci associated with chilling tolerance. Recently, several other loci have been examined using more advanced research resources, including near-isogenic lines and molecular markers. The genomic locations of these loci are summarized in Table 2.

Among the loci, locus *T/t* (tawny/gray pubescence) has been characterized most intensively. Yumoto and Tsuchiya[39] found that cultivars with tawny pubescence that also harbored a brown hilum encoded by the *i-i* allele at another independent locus displayed higher degrees of chilling tolerance than those of cultivars with gray pubescence that also harbored a yellow hilum encoded by the *I* allele. Takahashi and Asanuma[32] subsequently revealed by comparing near-isogenic lines using a method similar to method A that the locus associated with chilling tolerance was *T/t*. Superior chilling tolerance associated with the *T* allele was also proved in Canada in the field[15]. Recently, a line of evidence showing that the *T* allele encodes flavonoid-3'-hydroxylase (F3'H)[16,35,36] has been presented. The antioxidant activity of the product of F3'H is considered to contribute to conferring chilling tolerance[35], although direct evidence remains to be provided.

The *T* locus is closely linked to the *E1* locus, which is known as a major maturity locus among the eight loci controlling flowering and maturity time, *E1–E7* and *J*[19]. Since representative chilling tolerance cultivars such as Kitamusume were known to harbor the *T* and *E1* alleles, the *E1* locus also drew scientists' attention. Kurosaki et al.[14] used four near-isogenic lines segregating for the *T* and *E1* loci, namely, *T-E1*, *t-E1*, *T-e1*, and *t-e1*. A comparison of these lines under the growth chamber condition using method A and under field conditions revealed the advantage of the *E1* allele over the *e1* allele with regard to chilling tolerance as well as that of *T* over *t*. The association of maturity loci with chilling tolerance was also demonstrated using more complex near-isogenic lines[33]. Lines with two genotypes at multiple maturity loci, *E1e3e4* and *e1E3E4*, exhibit similar flowering and maturity times when they are grown in a field in Hokkaido. Chilling treatment with method A revealed a difference in seed yield between these lines, suggesting that maturity loci affect chilling tolerance not by merely avoiding exposure to low temperatures at sensitive stages[33].

The recent advent of molecular markers based on PCR technology for soybean genomics[2,25,37] has enabled QTL analysis for a population derived from a cross between relatively closely related accessions. The first QTL analysis of chilling tolerance in soybean was conducted using recombinant inbred lines derived from a cross between two cultivars from Hokkaido, Toyomusume and Hayahikari[6]. Using chilling treatment with method C, QTLs for chilling tolerance were detected in the genomic regions containing the *E1* and *T* loci and the *E3* locus in addition to a new locus with minor effect on linkage group H (Table 2). The QTL on linkage group H affected seed number per plant at a low temperature. Although the logarithm of odds scores of the region including the *E4* locus did not exceed the significant level, this region displayed epistatic interaction with that including the *E3*

Table 2. Genetic loci associated with chilling tolerance and their genomic location

Linkage Group[1)]	Position[2)]	Locus	Reference
B1	60	*APX1*	4
C2	110	*T*	6, 14, 15, 29, 30
	115	*E1*	14, 30
H	5	*Satt635*	6
I	40	*E4*	6, 30
	50	*Ln*	19
K	110	*P1*	19
L	90	*Dt1*	19
	100	*E3*	6, 30

1)2): The linkage group and the approximate position (cM) are based on the linkage map constructed by Song et al.[26]

locus. The Hayahikari genotype, *E1e3e4T*, appeared to have the effect of promoting chilling tolerance. These findings seemed to confirm the association of the *T* and maturity loci with chilling tolerance. However, the introduction of *E1e3e4* into Toyomusume by marker-assisted selection and backcrossing did not improve the chilling tolerance (H. Funatsuki and S. Ohnishi, unpublished data), suggesting the involvement of other loci near the maturity loci. Further investigation is needed to reach a conclusion.

Chilling tolerance for seed quality

Chilling temperatures at the early reproductive phase cause a brown pigmentation and cracking in the seed coat around the hilum (Fig. 2A)[26,27,29,31]. The unfavorable heat-resistant pigmentation reduces the commercial value with Japanese traditional cooking like "Nimame", a whole shape use. Cracking causes poor storage stability by allowing rapid entry of moisture and providing an easy access to microorganisms.

A method for evaluating browning in seed coats at low temperatures was developed by Oka et al.[18]. They found that immature seeds approximately eight days after anthesis are the most sensitive to low temperature. Ten-day treatment from this stage was sufficient to induce browning in seed coats in the chilling-sensitive genotype, while this treatment had little effect on pod and seed development. Using a similar method, Takahashi[28] revealed that the *T* and *I* alleles had the effect of reducing browning in seed coats in comparison with other alleles, *t* and *i-i*[28].

An association of maturity loci with chilling tolerance has been demonstrated for seed quality as well as for seed yield[1,7,30]. It is common with chilling tolerance for seed yield that the *E1* and *e4* alleles promoted tolerance for browning in seed coats, while the *e3* allele had a negative effect[30] in contrast to its positive effect on chilling tolerance for seed yield. In a comparison of two genotypes with similar flowering and maturity times, *E1e3e4* and *e1E3E4*, the former displayed a higher degree of chilling tolerance for seed quality[1]. It is possible that some genes are involved in chilling tolerance both for seed quality and for yield, while the presence of genes specifically responsible for either of the traits is also suggested by the fact that some cultivars such as Koganejiro exhibit differential degrees of tolerance for seed quality and for seed yield.

A genotypic difference in tolerance to seed coat cracking at a low temperature has also been reported[26]. The genotypes tolerant to browning in seed coats tend to be tolerant to seed coat cracking induced by low temperature[18]. For example, lines with the *E1*, *T*, *I*, and *e4* alleles are more tolerant than are those with other alleles[1,28,30]. Since these alleles are not associated with tolerance to cracking at ambient temperature induced by pod-removal treatment[38], the mechanism underlying cracking at low temperature may be different from that underlying cracking caused by the disturbance of the source-sink balance.

Recently, a landmark cultivar, Toyoharuka, that shows nearly complete tolerance to chilling stress in terms of browning in seed coats and hilums, was developed by TAES (Fig. 2B). Toyoharuka also exhibits a high degree of tolerance to seed coat cracking induced by low temperature (Fig. 2B), while this cultivar is ranked as being susceptible to seed coat cracking induced by pod-removal treatment. Preliminary genetic analysis has suggested the presence of a major genetic factor suppressing the browning both in seed coats and hilums (S. Ohnishi & M. Senda, unpublished results). The chilling tolerance appears to be independent of flowering time or maturity (S. Ohnishi & H. Funatsuki, unpublished results). The development of molecular markers for the gene is now in progress.

Concluding remarks

Drought and heat stress now seems to be attracting more attention than chilling tolerance because of the ever-increasing serious problem of global warming. However, chilling tolerance is still an important trait in cool areas since the sharp climatic fluctuation, which we have often experienced recently, could cause exposure of plants to low temperatures as well. Although remarkable progress in research on soybean chilling tolerance has been made during the past decade as described above, higher levels of chilling tolerance must be conferred to soybean to maintain stable yield and quality of the product. To achieve this goal, a better understanding of the genetic and physiological mechanisms underlying chilling injury and tolerance is needed. Chilling-tolerant cultivars such as Toyoharuka should be useful as plant materials in studies with such aims. In addition, some physiological events related to chilling injury are now understood at the molecular level[10,24]. Moreover, the preliminary whole genome sequence of soybean has been published[3]. Improved efficiencies in the production of transgenic soybeans have also been achieved[11,23]. Effective use of these research resources and information may result in elucidation of the mechanisms, which would lead to the detection or creation of novel useful genetic variations and the establishment of efficient selection

systems for chilling tolerance in soybean.

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References

1. Benitez, E. R. et al. (2004) Soybean maturity gene effects on seed coat pigmentation and cracking in response to low temperatures. *Crop Sci*., **44**, 2038–2042.
2. Cregan, P. B. (1999) An integrated genetic linkage map of the soybean genome. *Crop Sci*., **39**, 1464–1490.
3. DoE Joint Genome Institute (2008) DOE JGI releases soybean genome assembly. http://www.jgi.doe.gov/News/news_1_17_08.html.
4. Funatsuki, H. et al. (2003) Deficiency of a cytosolic ascorbate peroxidase associated with chilling tolerance in soybean. *Theor. Appl. Genet.*, **106**, 494–502.
5. Funatsuki, H. et al. (2004) Methods for evaluation of soybean chilling tolerance at the reproductive stage under artificial climatic conditions. *Plant Breed*., **123**, 558–563.
6. Funatsuki, H. et al. (2005) Mapping of QTL associated with chilling tolerance during reproductive growth in soybean. *Theor. Appl. Genet.*, **111**, 851–861.
7. Githiri, S. M. et al. (2007) QTL analysis of low temperature induced browning in soybean seed coats. *J. Hered.*, **98**, 360–366.
8. Greaves, J. A. (1996) Improving suboptimal temperature tolerance in maize – search for variation. *J. Exp. Bot*., **47**, 307–323.
9. Hashimoto, K. & Yamamoto, T. (1970) Studies on the cool injury in bean plants. I. Effect of nitrogen under low temperature on the pod setting and fertility of soybeans. *Proc. Crop Sci. Soc. Jpn*., **39**, 156–163.
10. Kasai, A. et al. (2007) Structural features of GmIRCHS, candidate of the I gene inhibiting seed coat pigmentation in soybean: implications for inducing endogenous RNA silencing of chalcone synthase genes. *Plant Mol. Biol.*, **64**, 467–479.
11. Kita, Y. et al. (2007) Genetic improvement of the somatic embryogenesis and regeneration in soybean and transformation of the improved breeding lines. *Plant Cell Rep.*, **26**, 439–447.
12. Kurosaki, H. & Yumoto, S. (2003) Effects of low temperature and shading during flowering on the yield components in soybeans. *Plant Prod. Sci*., **6**, 17–23.
13. Kurosaki, H., Yumoto, S. & Matsukawa, I. (2003) Pod setting pattern during and after low temperature and the mechanism of cold-weather tolerance at the flowering stage in soybeans. *Plant Prod. Sci*., **6**, 247–254.
14. Kurosaki, H., Yumoto, S. & Matsukawa, I. (2004) Correlation of cold-weather tolerance with pubescence color and flowering time in yellow hilum soybeans in Hokkaido. *Breed. Sci.*, **54**, 303–311.
15. Morrison, M. J. et al. (1997) Yield of cool-season soybean lines differing in pubescence color and density. *Agron. J*., **89**, 218–221.
16. Nagamatsu, A. et al. (2007) Functional analysis of soybean genes involved in flavonoid biosynthesis by virus-induced gene silencing. *Plant Biotechol. J*., **5**, 778–790.
17. Ohnishi, S. & Shirai, S. (2006) Sensitivity to low temperatures at the pollen development stage in soybean and the evaluation method. http://nics.naro.affrc.go.jp/seika/h18/d18009.html.
18. Oka, K. et al. (1989) Influence of the stage and the period of low temperature treatment on the generation of browning in seed coats of yellow-hilum soybeans. *Nihon Ikushugakkai Nihon Sakumotsu Gakkai Hokkaido Danwakai Kaiho (Rep. Hokkaido Branch Jpn. Soc. Breed. Crop Sci. Soc. Jpn.)*, **29**, 22 [In Japanese].
19. Palmer, R. G. et al. (2004) Qualitative genetics. *In* Soybeans: improvement, production, and uses, third edition, eds. Boerma, H. R. & Specht, J. E., ASA, CSSA, SSSA, Madison, 137–233.
20. Saito, M. (1972) Summary of researches for cold-weather damage in field crops. Soybean breeding for cold-weather damage. *Rep. Hokkaido Branch Jpn. Soc. Breed. Crop Sci. Soc. Jpn*., **13**, 63–65 [In Japanese].
21. Sanbuichi, T. (1979) Studies on cool weather tolerance in soybean breeding. *Hokkaidoritsu Nogyo Shikenjo Shuho (Rep. Hokkaido Prefect. Agric. Exp. Stn.)*, **28**, 1–57 [In Japanese with English summary].
22. Satake, T. & Hayase, H. (1970) Male sterility caused by cooling treatment at the young microspore stage in rice plants. V. Estimation of pollen developmental stage and the most sensitive stage to coolness. *Proc. Crop Sci. Soc. Jpn*., **39**, 468–473.
23. Sato, H. et al. (2007) Production of transgenic plants and their early seed set in Japanese soybean variety, Kariyutaka. *Plant Biotechnol*., **24**, 533–536.
24. Senda, M. et al. (2004) Patterning of virus-infected *Glycine max* seed coat is associated with suppression of endogenous silencing of chalcone synthase genes. *Plant Cell*, **16**, 807–818.
25. Song, Q. J. et al. (2004) A new integrated genetic linkage map of the soybean. *Theor. Appl. Genet*., **109**, 122–128.
26. Srinivasan, A. & Arihara, J. (1994) Soybean seed discoloration and cracking in response to low temperatures during early reproductive growth. *Crop Sci*., **34**, 1611–1617.
27. Sunada, K. and Ito, T. (1982) Influence of low-temperature treatment during growth on seed quality in soybean (discoloration and cracking of seed coat around hilum). *Rep. Hokkaido Branch Jpn. Soc. Breed. Crop Sci. Soc. Jpn*., **22**, 34 [In Japanese].
28. Takahashi, R. (1997) Association of soybean genes *I* and *T* with low-temperature induced seed coat deterioration. *Crop Sci*., **37**, 1755–1759.
29. Takahashi, R. & Abe, J. (1994) Genetic and linkage analysis of low temperature-induced browning in soybean seed coats. *J. Hered*., **85**, 447–450.
30. Takahashi, R. & Abe, J. (1999) Soybean maturity genes associated with seed coat pigmentation and cracking in response to low temperatures. *Crop Sci*., **39**, 1657–1662.
31. Takahashi, R. & Akiyama, T. (1993) Characterization of a melanin associated with low temperature-induced browning in soybean seed coats. *Phytochemistry*, **34**, 587–588.

32. Takahashi, R. & Asanuma, S. (1996) Association of *T* gene with chilling tolerance in soybean. *Crop Sci*., **36**, 559–562.
33. Takahashi, R. et al. (2005) Soybean maturity and pubescence color genes improve chilling tolerance. *Crop Sci*., **45**, 1387–1393.
34. Tanaka, Y. et al. (2003) A new soybean variety "Yukihomare". *Rep. Hokkaido Prefect. Agric. Exp. Stn*., **84**, 13–24 [In Japanese with English summary].
35. Toda, K. et al. (2002) A single-base deletion in soybean flavonoid 3'-hydroxylase gene is associated with gray pubescence color. *Plant Mol. Biol*., **50**, 187–196.
36. Toda, K. et al. (2005) Structure of flavonoid 3 '-hydroxylase gene for pubescence color in soybean. *Crop Sci*., **45**, 2212–2217.
37. Yamanaka, N. et al. (2001) An informative linkage map of soybean reveals QTLs for flowering time, leaflet morphology and regions of segregation distortion. *DNA Res*., **8**, 61–72.
38. Yang, D. et al. (2002) Seed coat cracking in soybean isolines for pubescence color and maturity. *Crop Sci*., **42**, 71–75.
39. Yumoto, S. & Tsuchiya, T. (1991) Response to low temperature and its genotypic variations in soybean cultivars. *Rep. Hokkaido Branch Jpn. Soc. Breed. Crop Sci. Soc. Jpn*., **31**, 60 [In Japanese].
40. Yumoto, S. et al. (2000) A new soybean variety "Hayahikari". *Rep. Hokkaido Prefect. Agric. Exp. Stn*., **78**, 19–37 [In Japanese with English summary].