

Characterization of a Novel Early Leaf-Senescence Line in Common Buckwheat (*Fagopyrum esculentum*)

Katsuhiro MATSUI^{1*}, Kiyofumi MORI², Yumiko KOGA³, Yukiko FUKUDA⁴, Yoshihiko FURUTA³, Asana MATSUURA⁴ and Takahisa TETSUKA¹

¹ National Agriculture and Food Research Organization (NARO), Kyushu Okinawa Agricultural Research Center (KARC) (Koshi, Kumamoto 861-1192, Japan)

² Osumi Branch, Kagoshima Prefectural Institute for Agricultural Development (Kanoya, Kagoshima 893-1601, Japan)

³ Applied Biological Sciences, Gifu University (Gifu, Gifu 501-1193, Japan)

⁴ School of Agriculture, Tokai University (Aso, Kumamoto 869-1404, Japan)

Abstract

Seeds of common buckwheat usually mature while leaves are still green, causing problems for harvest machinery and limiting yields. We developed an early leaf-senescence line, Kyushu 1, from spontaneous mutants. To characterize this mutant line physiologically and genetically, we measured the change in leaf chlorophyll content in two regions of Japan with different environments, and performed genetic analyses with self-compatible lines. The chlorophyll content of Kyushu 1 was lower than that of the wild type at seed maturity in both regions. F₁ progeny of the cross between wild-type plants and Kyushu 1 showed a wild-type phenotype. F₂ progeny segregated into wild-type and early leaf-senescence phenotypes in a 3:1 or 13:3 ratio, suggesting that the early leaf senescence of Kyushu 1 is controlled by either a single recessive gene or an epistatic interaction between two loci.

Discipline: Plant breeding

Additional key words: chlorophyll, genetic analysis, mutant, SPAD

Introduction

Leaf senescence is the final stage of leaf development and is considered a form of programmed cell death. A color change from green to yellow, orange, or red is a typical sign of senescence due to chlorophyll degradation¹³. The visible yellowing and chlorophyll loss are widely taken as a sign of the progression of senescence in many plants, and correlate with other biochemical changes that occur during leaf senescence^{6,10}.

Common buckwheat (*Fagopyrum esculentum*) is grown widely around the world, and its flour is used in foods such as noodles and cakes. The seeds usually mature before the leaves senesce, notably in warm areas. Mature seeds start to abscise before later-formed seeds mature, but the rate of seed shattering is affected by environmental conditions². The peak yield is obtained when the seed discoloration rate is 75 to 90%^{2,4}, whereas many green leaves remain when the seed discoloration rate is around 80%. Buckwheat is usually harvested with a combine harvester, but the many

remaining green leaves and stems impede the sorting of seeds. Although maximizing the yield is important, the presence of many green leaves makes it difficult for farmers to decide when to harvest. Furthermore, delaying the harvest time increases potential for preharvest sprouting in response to rain, consequently impairing the flour quality⁵.

Clarifying the mechanism of leaf senescence is important not only for science, but also agriculture, e.g. in improving yields¹⁵. Many genetic mutants with delayed or early leaf senescence have been reported in many plants⁹, and are used to clarify the molecular basis of the leaf senescence mechanism and improve agricultural traits such as yield^{15,16}. Here, we describe a new early leaf-senescence mutant of common buckwheat and characterize its traits physiologically and genetically.

Materials and methods

1. Plant materials

Seven early leaf-senescence mutants that seemed homozygous for genes controlling early leaf senescence

*Corresponding author: e-mail matsuik@affrc.go.jp

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were found in a breeding population (~1000 plants) produced from a cross between Hashikami-wase (intermediate maturity) and Kurino-zairai (autumn maturity), neither of which has the trait. By recurrent selection (F_{10}) of populations derived from the seven mutants, we developed an early leaf-senescence line, Kyushu 1 (KS1). We analyzed the chlorophyll contents of KS1 (intermediate maturity) and two wild-type common buckwheats, Hitachi-akisoba (HTC), a leading cultivar in the Kanto area of Japan (intermediate maturity), and Kanoya-zairai (KNY), a leading cultivar in southern Kyushu (autumn maturity).

2. Evaluation of chlorophyll contents under different environmental conditions

We designed our experiments to clarify how the early leaf-senescence trait of KS1 manifested in different places and years, and whether the timing of senescence differed by leaf age (nodal position). We analyzed the chlorophyll contents of KS1, HTC, and KNY over 6 weeks in two experimental fields, one at the NARO Kyushu Okinawa Agricultural Research Center (NARO/KARC), in the middle of Kyushu, and the other at the Osumi Branch of the Kagoshima Prefectural Institute for Agricultural Development (KIAD), in southern Kyushu. We used a hand-held chlorophyll meter (SPAD-502, Minolta, Japan) to measure the relative chlorophyll contents of leaves *in situ* as an index of leaf senescence, based on a correlation between the chlorophyll content per unit leaf area and the optically measured greenness of intact leaves^{7,8}. We averaged readings from three positions on each leaf. In the experiment at NARO/KARC, we measured three leaves per plant (upper, mid-plant, and basal positions) of all three cultivars in three plants in 2004 and four plants in 2006. In the experiment at KIAD, we measured the upper leaves of 20 randomly selected plants sown early or late, assessing KNY and KS1 in 2005 and all three in 2006.

3. Estimation of the number of genes controlling early leaf-senescence

To estimate how many genes control the early leaf-senescence trait in KS1, we performed a genetic analysis with self-compatible lines. Buckwheat is a sporophytic-heteromorphic, self-incompatible species, which requires insects to cross-pollinate between pin and thrum flower forms^{1,3}. In previous research we developed self-compatible (sco) lines^{11,12}, so we used two—sco-Asahimura-zairai 3 (sco-ASH) and sco-KNY—to produce segregating populations. Sco-ASH was produced by backcrossing Asahimura-zairai 3 (ASH; intermediate maturity) with the self-compatible line ‘Buckwheat Norin-PL1’¹². Sco-KNY was produced by backcrossing KNY with ‘Buckwheat Norin-PL1’. We produced segregating populations by making crosses between sco-ASH and KS1 (06AL1213) and

between sco-KNY and KS1 (06AL1617). F_2 segregating plants were obtained by the self-pollination of F_1 plants. We measured the chlorophyll content of an upper leaf of segregating populations 1 week after maturation (defined here as when 80% of seeds turn black).

Results

1. Morphological and physiological characterization of the early leaf-senescence mutants

At Kumamoto, the plant height of KS1 was about 110 cm and the number of primary branches was 3–5 (Fig. 1). Seeds of KS1 matured on 16 November in 2004 and 24 October in 2006 in Kumamoto (Table 1). The maturity dates were close to those of HTC. The seeds of KS1 and HTC matured in both tests in Kumamoto, but those of KNY did not mature in either test. The ecotype of HTC is classified as intermediate, so the ecotype of KS1 should be the same.

At Kagoshima, seeds of KS1 sown on 13 and 22 September, 2005 matured on 16 and 29 November, respectively, while those sown on 4 and 20 September, 2006 matured on 2 and 17 November, respectively.

2. Evaluation of chlorophyll contents under different environmental conditions

At Kumamoto in 2004, the chlorophyll degradation of leaves of KS1 started between 12 and 19 October in basal leaves, and 19 and 26 October in mid-plant and upper leaves (Fig. 2). In 2006, it started between 10 and 17 October in both mid- and upper positions, a little later than in mid-plant leaves (Fig. 2). These results indicate that the degradation of chlorophyll occurred almost simultaneously in all leaves

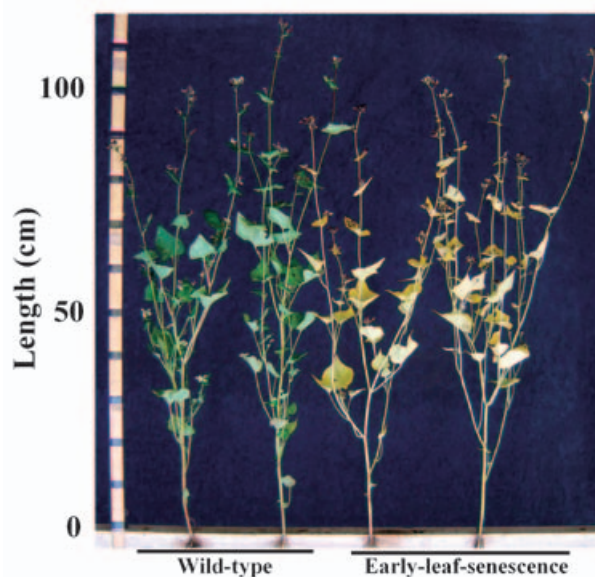


Fig. 1. Difference in leaf senescence at seed maturity between wild-type and early leaf-senescence plants

Table 1. Average chlorophyll contents (SPAD value) and maturity dates

Place	Year	Sowing date	Line	Maturity date	SPAD *						
					I	II	III	IV	V	VI	VII
Kumamoto	2004	September 7	HTC	November 13	36.0 a	37.0 a	37.7 a	35.7 a	33.0 a	32.1 a	28.1 a
			KNY	NM	34.0 a	35.3 a	38.7 a	36.0 a	36.5 a	34.7 a	33.0 a
			KS1	November 16	30.1 a	31.3 a	29.5 b	22.2 b	16.2 b	13.3 b	7.4 b
	2006	August 29	HTC	October 24	31.0 a	37.4 a	40.5 a	41.4 a	35.1 a	27.1 ab	20.0 a
			KNY	NM	35.7 a	35.7 a	39.7 a	43.4 a	41.6 a	33.1 a	24.4 a
			KS1	October 24	31.7 a	34.9 a	36.2 a	37.4 a	30.6 a	20.1 b	5.2 b
Kagoshima	2005	September 13	KNY	December 7	32.8 a	33.0 a	34.1 a	34.1 a	33.7 a	32.8 a	30.6 a
			KS1	November 16	30.7 b	28.2 b	28.4 b	19.2 b	16.7 b	9.0 b	7.3 b
		September 22	KNY	December 14	31.2 a	28.5 a	32.8 a	36.1 a	35.9 a	35.1 a	32.4 a
	KS1		November 29	25.4 b	24.2 b	24.2 b	22.5 b	19.3 b	14.6 b	11.4 b	
	2006	September 4	HTC	October 27	35.8 a	38.6 a	36.5 a	31.1 ac	28.6 a	23.7 a	20.8 a
			KNY	November 9	35.6 a	39.0 a	43.1 b	37.7 b	33.5 b	30.8 b	22.9 a
			KS1	November 2	30.9 b	33.8 b	31.7 c	27.9 c	19.8 c	17.1 c	11.3 b
	September 20	HTC	November 13	37.2 a	40.5 a	38.8 a	30.8 b	25.6 a	22.4 a	21.3 a	
		KNY	November 28	37.7 a	40.0 a	39.5 a	35.9 a	28.8 ab	26.5 a	25.1 a	
KS1		November 17	34.2 b	36.0 b	33.8 b	24.5 c	17.5 b	15.5 b	14.6 b		

I–VII represent the measurement dates shown in Figs. 2 and 3.

HTC, Hitachi-akisoba; KNY, Kanoya-zairai; KS1, Kyushu 1. NM, not matured.

* Means followed by the same letter within a column among lines do not differ significantly at $P < 0.05$ by the Tukey–Kramer test.

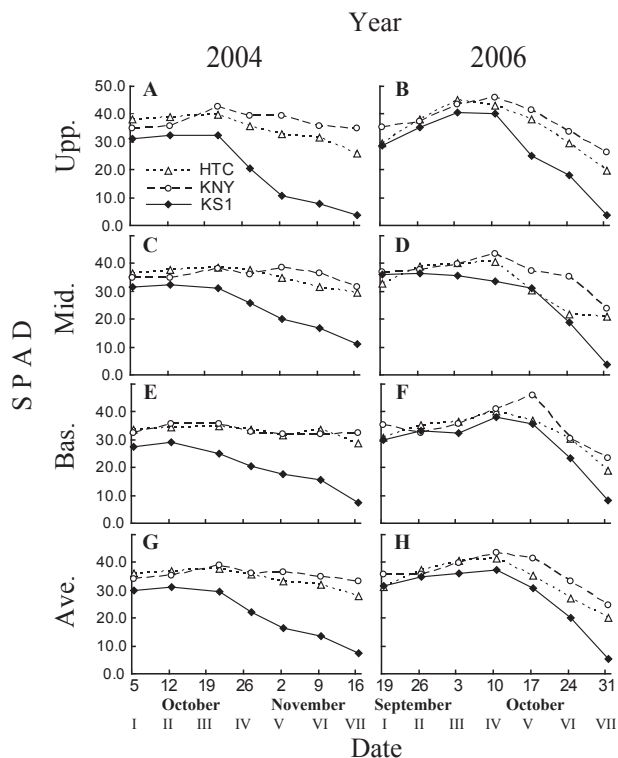


Fig. 2. Changes in SPAD (chlorophyll) values during the senescence of KS1 (KS1) and control Hitachi-akisoba (HTC) and Kanoya-zairai (KNY) in Kumamoto

Upp., upper leaves; Mid., mid-plant leaves; Bas., basal leaves; Ave., average. I–VII refer to the measurement dates in Table 1.

irrespective of leaf age (Fig. 2).

The average chlorophyll contents of KS1 were significantly lower than those of KNY and HTC from 19 October to 16 November, 2004, and significantly lower than those of KNY on 24 and 31 October and HTC on 31 October, 2006 (Table 1). Although the seeds of KS1 matured on 16 November, later than HTC at Kumamoto in 2004, the chlorophyll content of KS1 at seed maturity was lower than that of HTC in both years.

At Kagoshima in 2005, we tested whether the sowing date affected leaf senescence. The chlorophyll content of KS1 decreased over time. Conversely, KNY showed no notable degradation in leaf color between 17 October and 28 November in either sowing date block (Figs. 3A, C), while the chlorophyll contents of KS1 were significantly lower than those of KNY between 17 October and 28 November.

In 2006, we included HTC, because KNY matures late, and we wanted to know whether the early leaf senescence of KS1 was caused by early maturation. The chlorophyll contents peaked on 18 October in KNY and on 11 October in HTC and KS1 in the early sown block, and on 26 October in all three in the late-sown block. Although the HTC seeds matured earlier than those of KS1 (Table 1), the chlorophyll content of HTC on 23 November exceeded that of KS1, indicating that many green leaves remained. The chlorophyll contents of KS1 were significantly lower than those of KNY and HTC between 4 October and 15 November

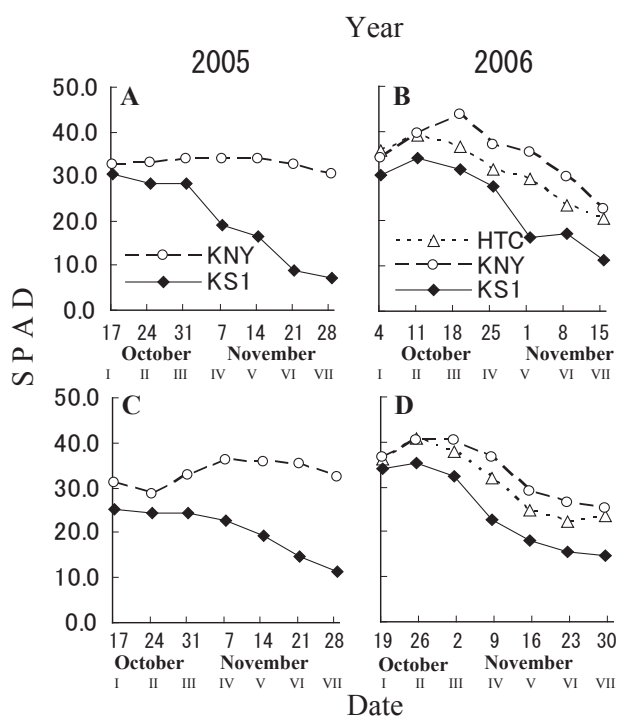


Fig. 3. Changes in SPAD (chlorophyll) values during the senescence of KS1 (KS1) and control Hitachi-akisoba (HTC) and Kanoya-zairai (KNY) in Kagoshima

A, B, early sowing; C, D, late sowing (Table 1). I–VII refer to the measurement dates in Table 1.

(except HTC on 25 October) in the early sown block (Fig. 3B, Table 1), and between 19 October and 30 November (except KNY on 16 November) in the late-sown block (Fig. 3D, Table 1).

3. Estimation of number of genes controlling early leaf-senescence

To reveal the inheritance of the early leaf-senescence, we performed genetic analysis using self-compatible plants. Self-incompatibility and self-compatibility are controlled by the *S* locus, and the self-compatibility allele *S^h* dominates the self-incompatibility allele, *s^{17,11}*. *F*₁ plants were produced by a cross between early leaf-senescence self-incompatible pin plants of KS1 and wild-type self-compatible long-homostyle plants of sco-ASH and sco-KNY. All six *F*₁ plants resulting from each cross showed a wild-type phenotype. The *F*₂ populations of each cross were tested for segregation of chlorophyll content. Two peaks appeared (Fig. 4), although the peak in the KS1 × sco-KNY *F*₂ population is unclear due to the small population size. We considered the interval of lowest frequency between these two peaks as the boundary between the early senescence and wild-type groups. The boundary intervals corresponded to a SPAD value of 16–18 in the KS1 × sco-ASH *F*₂ population

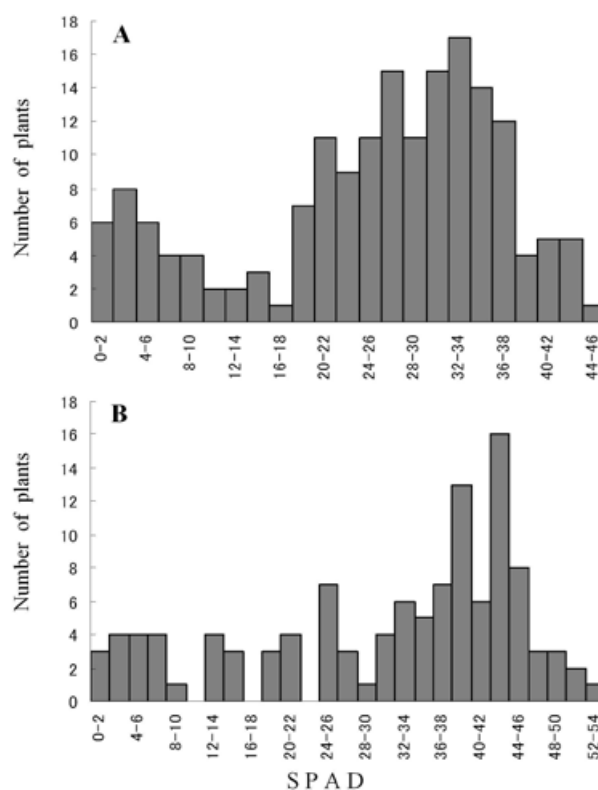


Fig. 4. Histogram showing SPAD (chlorophyll) value distribution in *F*₂ populations of (A) sco-ASH × KS1 and (B) sco-KNY × KS1 crosses

and one of 10–24 in the KS1 × sco-KNY *F*₂ population. We classified the *F*₂ progeny of both crosses accordingly and performed a segregation analysis. The segregation between the wild-type (SPAD > 17 in both populations) and early leaf-senescence phenotypes (SPAD < 17 in both populations) fitted both 3:1 and 13:3 ratios in both populations (Table 2), suggesting that the early leaf-senescence characteristic is controlled by either a single recessive gene or an epistatic interaction between two loci.

Discussion

The leaves and stems of buckwheat should ideally mature as fast as possible after seed coloration reaches 80%. In our trials, the leaves and stems of KS1 senesced early at both test sites, and most leaves senesced simultaneously, two traits that facilitate harvesting. To our knowledge, this is the first report of an early leaf-senescence mutant of common buckwheat. Many genetic mutants with delayed or early leaf-senescence have been reported in many plants⁹. One trait often manipulated to improve yield is delayed leaf senescence¹⁴, but for buckwheat cultivation, the opposite trait may avoid the problem encountered by the combine harvester, because leaves and stems should ideally mature

Table 2. Genetic analysis of early leaf senescence with self-compatible lines

Line	No. of progeny	Flower morphology (long-homostyle vs. pin)				Leaf color (green, SPAD>17 vs. yellow, SPAD<17)						
		Observed	Expected	χ^2	<i>P</i>	Observed	Expected	χ^2	<i>P</i>			
06AL(12-13)	173	128:45	15:1	115.302	**	138:35	15:1	57.714	**			
			9:7	22.120	**					9:7	38.884	**
			13:3	5.988	*					13:3	0.249	0.50 < <i>P</i> < 0.70
			3:1	0.094	0.70 < <i>P</i> < 0.80					3:1	2.098	0.05 < <i>P</i> < 0.10
06AL(16-17)	115	88:27	15:1	58.254	**	92:23	15:1	37.106	**			
			9:7	19.203	**					9:7	26.358	**
			13:3	1.688	0.10 < <i>P</i> < 0.20					13:3	0.118	0.50 < <i>P</i> < 0.70
			3:1	0.142	0.70 < <i>P</i> < 0.80					3:1	1.533	0.20 < <i>P</i> < 0.30

* Significant at *P* = 0.05, ** Significant at *P* = 0.01.

as fast as possible after the seed coloration reaches 80%, and senescence should cause leaf abscission and reduce the moisture contents of leaves and stems. Furthermore, a clear distinction of the leaf color showing the early leaf-senescence trait at maturity could aid farmers in deciding when to harvest.

Our results show that early leaf-senescence in KS1 is controlled by either a single recessive gene or an epistatic interaction between two loci. Control of the early leaf-senescence trait by a small number of genes facilitates the selection of early leaf-senescence plants with other desirable agricultural traits, such as high yields, lodging resistance, and disease resistance, while molecular markers such as SSR markers linked to the genes controlling the trait could help us develop new cultivars.

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