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Suitability of Rice-Tartary Buckwheat for Crossbreeding and for Utilization of Rutin

Yuji MUKASA*, Tatsuro SUZUKI and Yutaka HONDA

Local Crop Breeding Team, National Agricultural Research Center for Hokkaido Region, National Agriculture and Food Research Organization (Memuro, Hokkaido 082–0081, Japan)

Abstract

Rice-Tartary buckwheat is a form of Tartary buckwheat grown and used in place of rice in limited areas of Nepal, Bhutan, and southern China. It has a non-adhering hull that splits longitudinally in three, unlike other Tartary buckwheats, which have an adhering hull that is hard to remove. Information on rice-Tartary buckwheat is limited, and its suitability for crossbreeding is unclear. We reciprocally crossed Tartary and rice-Tartary buckwheats, and backcrossed rice-Tartary-type progeny and Tartary buckwheat. Hybridization using hot-water emasculation was successful, and over half of the hand-pollinated flowers set mature seeds. Segregation analyses revealed that the non-adhering hull is controlled by a single recessive gene. F_2 segregates showed almost no relation between the non-adhering hull and earliness, suggesting that selection for early-maturing non-adhering-hulled plants is feasible. Progeny analysis of a cross between rice-Tartary-type plants and plants with a dark red cotyledon suggested no linkage between the two controlling loci. The rutin concentration in dehulled grain was stable after immersion in water, although that in flour rapidly decreased after the addition of water. Rice-Tartary buckwheat is suitable for crossbreeding with Tartary buckwheat, and the trait of non-adhering hull will allow the use of dehulled grain as a dietary source of rutin.

Discipline: Genetic resources
Additional key words: Dark red cotyledon, Dehulled grain, Earliness, Hull color, Non-adhering hull

Introduction

Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) is a cultivated, self-pollinating species of buckwheat that accumulates large amounts of rutin in its seeds[2]. Rutin is a flavonoid with blood-vessel protective, antioxidant[4], and antihypertensive[3] activities. Rice-Tartary is a form of Tartary buckwheat with uncertain taxonomic status with a non-adhering hull that splits longitudinally in three, unlike other Tartary buckwheats, which have an adhering hull that is hard to remove. The seeds of rice-Tartary buckwheat can be easily dehulled by rubbing between the fingers. Rice-Tartary buckwheat is grown and used in place of rice in limited areas of Nepal, Bhutan, and southern China[1]. Wang and Campbell[10] were the first to report the hybridization of rice-Tartary and Tartary buckwheats for developing Tartary buckwheat that possesses non-adhering hull, but they obtained only a few hybrids, and backcrossing failed. Information on rice-Tartary buckwheat for sustainable crossbreeding is limited, and its crossability with Tartary buckwheat remains unclear. We have developed a simple method of emasculating Tartary buckwheat that simplifies hybridization[5]. Here, we investigated the crossability of rice-Tartary and Tartary buckwheats and backcrosses using our method.

As Wang and Campbell[10] described, rice-Tartary buckwheat that we possessed had a long vegetative period and excessive plant growth in our experimental field. A long vegetative period is a critical defect at high latitudes because the plant is always killed by autumn frosts before maturation. Therefore, our primary target in breeding is earliness. Here we examined the relation between the non-adhering hull and extreme lateness through progeny analysis of the F_2 plants derived from the cross between extremely late-maturing rice-Tartary and early Tartary (cv. 'Hokkai T8').

The trait of non-adhering hull may be useful for seed sprout production of Tartary buckwheat. In commercial sprout production, the hull often remains on the developed cotyledon, causing complaints among consumers. Recently, we developed a new cultivar for seed sprout

*Corresponding author: e-mail mukasa@affrc.go.jp
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production, which has a distinctive dark red cotyledon (caused by a high anthocyanin concentration[7]), but it still has an adhering hull. The trait of non-adhering hull should be introduced for further improvement. Here we investigated the relation between the traits of non-adhering hull and dark red cotyledon through progeny analyses.

A dehulled grain may offer the advantage of avoiding enzymatic rutin degradation. Yasuda et al.[11] reported that Tartary buckwheat flour had high activity of a rutin-degrading enzyme, and the addition of water to the flour caused rapid decomposition of the rutin to quercetin. Suzuki et al.[8] found rutin mainly in the embryo of ripe seeds, but flavonol-3-glucosidase, a rutin-degrading enzyme, mainly in the testa, and confirmed this in germinated seedlings[9]. These results suggest that the rutin-degrading enzyme is largely isolated from rutin in seeds and that enzymatic rutin degradation may be avoidable by using dehulled grain for cooking. Here we investigated the rutin concentrations in dehulled grain after the addition of water.

Materials and methods

1. Parents, crossing, and planting for progeny analyses

The seeds of rice-Tartary buckwheat ('RT'), with a dark and non-adhering hull, were kindly provided to us by Kyoto University, Japan. The seeds are small and the yield is very low, mainly owing to the long vegetative period[10]. 'Hokkai T8', with a light-brown adhering hull, is a high-yielding cultivar of Tartary buckwheat grown widely in Hokkaido, Japan. It had been bred through pure line selection of 'Rotundatum', introduced from the former USSR. We carried out reciprocal crosses between 'RT' and 'Hokkai T8'.

In 2006, 'RT' was planted in pots on March 1 and 'Hokkai T8' on March 27. They were grown in a greenhouse until adequate numbers of flower buds were ready to use for crossing. The greenhouse was heated above 15°C until the middle of May and the day length was not controlled. Emasculation and hand pollination were carried out according to the method we developed[5]. The cross of 'RT' × 'Hokkai T8' (cross number 06T06) was carried out on May 10 and the reciprocal cross (06T07) on May 16. Mature seeds were harvested about 25 days after pollination and dried in a drying oven at 35°C for several days.

The F_1 seeds were planted in pots on July 5 and grown in a greenhouse until maturity. F_2 seeds were harvested from each F_1 plant on October 19. Two F_2 populations (06T06-1 and 06T07-1) were planted in a greenhouse in 2006 and the other F_2 populations were planted in an experimental field (latitude 42°53′N, longitude 143°4′E) in 2007. In the field, seeds were hand-planted on June 6 in two rows (5 cm apart) in one hill (60 cm wide) spaced 5 cm apart within the rows. Basal fertilizer was applied at a standard level corresponding to N 1.8 g/m^2, P_2O_5 7.2 g/m^2 and K_2O 4.2 g/m^2. Supports were installed in the middle of the growing season to avoid lodging. Plants were individually harvested once a week from August 28 to October 9 when about 90% of seeds on the main stem had turned a mature color. Frost prevented further investigation of maturing time, and the remaining plants were harvested on October 16. The plants were categorized into four groups according to the combination of hull type (adhering versus non-adhering) and hull color (dark versus light brown). Chi-square analysis was used to examine the fit of the observed segregations in hull type and hull color to the expected ratios. Natural day length ranged from 11 to 15.5 h during the experiment.

F_3s derived from 06T06-1 and 06T07-1 were grown in the field as above in 2007. The date of maturity, hull color, and 1000-seed weight were investigated in bulk.

2. Further crossing between rice-Tartary-like progeny and Tartary buckwheat

Several progeny having non-adhering hull (F_2 or F_3 plants) were crossed with Tartary buckwheat ('Hokkai T8', 'Yugoslavian (local)', 'Rotundatum', and 'IRB-FT-41') through the use of hot-water emasculation. F_2 plants were used as male after we checked the set seeds, and F_3 plants as female. Hand-pollination was carried out on March 15, May 2, and May 9 in a greenhouse according to the readiness of plants. The degree of crossability was calculated as the number of mature seeds divided by the number of hand-pollinated flowers.

3. Progeny analysis of cross between rice-Tartary and dark-red cotyledon cultivar

Two F_3 plants having non-adhering hull (06T07-1-1g-1g and 06T07-1-1g-2g) were used as the female parents and one cultivar with the gene for dark-red cotyledon (cv. 'Hokkai T10') was used as the male parent. Seeds were planted in pots on March 5, and crosses were carried out on April 16 (cross numbers 07T11 and 07T12). The F_1 seeds were planted in pots on May 24 and F_2 seeds were harvested from each F_1 plant on September 14. Fifty seeds of each F_2 population were planted in a square planter (60 cm × 60 cm), and segregations in cotyledon color and hull type were investigated. Chi-square analysis was used to examine the fit of the observed segregations to the expected ratios. All plants were grown in a greenhouse as above.

4. Determination of rutin concentration in dehulled grain in water

One F_3 line (06T07-1-1g-0) harvested in bulk was used. The grain was manually dehulled, and a portion of dehulled grain was ground with a mortar and pestle to make a control flour sample. Approximately 200 mg of flour or dehulled grain was weighed and put into a test tube with 0.5 mL of distilled water and then vortexed. The flour mixed with water was stood for 0, 10, 20, and 30 min and the dehulled grain mixed with water was stood for 30 min, 1, 2, 4, 8, and 24 h at room temperature. Five milliliters of methanol containing 1% phosphoric acid [1.1% (vol./vol.)] was added, and the mixture was immediately homogenized with a hand homogenizer (Ultra-Turrax T8, Ika-Werke Co., Germany) for 20 s at maximum speed. The homogenate was kept in a constant-temperature chamber at 40°C for 3 h and then filtered through a 0.5-µm PTFE membrane (Iwaki Co. Ltd., Japan). The rutin concentration was determined by the HPLC method of Suzuki et al.[8]. Four replicated samples were prepared and analyzed for each treatment.

Results

1. Crossability between rice-Tartary and Tartary buckwheats

We obtained six mature seeds from 'RT' × 'Hokkai T8' and 17 from the reciprocal cross. Although we did not count the hand-pollinated flowers, about half set mature seeds. In further backcrosses between rice-Tartary-type progeny and Tartary buckwheat, mature seeds were consistently obtained in both reciprocal crosses. Over half of the hand-pollinated flowers set mature seeds on average (Table 1). Their hybridity was confirmed by judging of the hull features of F_1 plants (data not shown). There were no difficulties in hybridization between rice-Tartary and Tartary buckwheats using the hot-water emasculation method.

2. Inheritance of non-adhering hull and relations with hull color and earliness

We established two F_2 populations from single F_1

Table 1. Crossability between Tartary and rice-Tartary progeny

Female	Male	No. of pollinated flowers (a)	No. of mature seeds (b)	(b)/(a) (%)
Tartary × Rice-Tartary				
Hokkai T8	06T06-1-4g	15	5	33.3
Hokkai T8	06T06-1-5g	14	12	85.7
Hokkai T8	06T06-1-6g	10	4	40.0
Hokkai T8	06T06-1-7g	4	3	75.0
Subtotal		43	24	55.8
Rice-Tartary × Tartary				
06T07-1-1g-1g	Hokkai T8	9	5	55.6
06T07-1-1g-2g	Hokkai T8	8	4	50.0
06T07-1-1g-3g	Yugoslavian (local)	8	3	37.5
06T07-1-1g-4g	Yugoslavian (local)	6	5	83.3
06T07-1-1g-5g	Rotundatum	8	6	75.0
06T07-1-1g-6g	Rotundatum	9	4	44.4
06T07-1-1g-7g	IRBFT-41	6	5	83.3
06T07-1-1g-8g	IRBFT-41	9	7	77.8
06T07-1-1g-9g	IRBFT-41	9	4	44.4
06T07-1-1g-10g	IRBFT-41	6	4	66.7
Subtotal		78	47	60.3
Total		121	71	58.7

Crosses were carried out by using hot water emasculation.

plants of 'RT' × 'Hokkai T8' (06T06-1 and 2) and 10 F_2 populations from the reciprocal cross (06T07-1 to 10). The other F_1 seeds did not germinate or were killed by diseases such as damping-off. The hulls of F_2 seeds on the F_1 plants were all dark and adhering in both reciprocal crosses, suggesting that non-adhering and light brown are recessive traits.

In the F_2 generation (F_3 seeds), the segregation patterns fitted the 3:1 ratio of adhering hull to non-adhering hull and the 3:1 ratio of dark hull to light brown hull, suggesting that both traits are controlled by single recessive genes (Table 2). These results agree with previous reports[6,10]. The segregation pattern also fitted 9:3:3:1 ratio of [adhering/dark] : [adhering/light brown] : [non-adhering/dark] : [non-adhering/light brown] in the dihybrid model, suggesting that the non-adhering hull is independent of hull color.

Figure 1 shows the distribution of maturation time in the parents and F_2 populations of 'Hokkai T8' × 'RT' (total of 06T07-2 to 10). The F_2 plants were categorized into the four groups listed above. The distribution patterns were roughly the same as each other, and extremely late-maturing plants with the same maturation time as 'RT' (date of maturation 'Res.') showed the same 9:3:3:1 segregation ratio as above. However, early-maturing F_2 plants with a non-adhering hull matured a little later than those with an adhering hull in both hull colors.

The maturation dates, hull colors, and 1000 seed weights of F_3 lines are shown in Table 3. All F_3 lines had the non-adhering hull, because we had selected the F_2 plants for their non-adhering hull. Several early-maturing lines such as 06T06-1-1g were obtained, although we did not select the F_2 plants for earliness as they had been grown in a greenhouse during winter. The maturity of the lines harvested on September 12 was relatively uniform, suggesting that the development of a rice-Tartary cultivar that matures before the onset of frosts is feasible. For reference, the average date of the first frost is October 8 in our area.

3. Relation between non-adhering hull and dark red cotyledon

Table 4 shows the segregation pattern of hull type and cotyledon color in the F_2 populations. The segregation pattern fitted the 9:3:3:1 ratio of [adhering/green cotyledon] : [adhering/dark red cotyledon] : [non-adhering/

Table 2. Segregation patterns of hull types (adhering versus non-adhering) and hull colors (dark versus light brown) in F_2 plants

Population	Number of plants				χ^2	P	χ^2	P	χ^2	P
hull type	Adhering		Non- adhering		for		for		for	
hull color	D[1)] hull	LB[2)] hull	D hull	LB hull	A[3)]:N[4)]=3:1		D:LB=3:1		9:3:3:1	
'RT'× 'Hokkai T8'										
06T06-1	55	22	16	5	0.67	0.41	0.34	0.56	1.23	0.75
06T06-2	166	56	47	21	0.37	0.54	0.37	0.54	1.56	0.67
Total	221	78	63	26	0.88	0.35	0.67	0.41	1.85	0.60
'Hokkai T8' × 'RT'										
06T07-1	36	17	11	0	2.08	0.15	0.08	0.77	6.17	0.10
06T07-2	90	24	23	10	0.51	0.48	0.27	0.60	1.93	0.59
06T07-3	93	31	24	6	2.50	0.11	0.08	0.78	2.81	0.42
06T07-4	87	31	25	11	0.22	0.64	0.42	0.51	0.87	0.83
06T07-5	84	25	29	11	0.27	0.60	0.06	0.81	0.66	0.88
06T07-6	89	31	20	14	0.70	0.40	1.46	0.22	4.94	0.18
06T07-7	96	26	23	13	0.41	0.52	0.01	0.92	3.49	0.32
06T07-8	86	26	33	10	0.62	0.43	0.62	0.43	0.88	0.83
06T07-9	84	26	24	10	0.15	0.70	0.00	1.00	0.59	0.90
06T07-10	40	13	16	3	0.07	0.79	0.30	0.59	0.99	0.80
Total	785	250	228	88	0.11	0.74	0.00	0.99	3.55	0.31

1): Dark hull, 2): Light brown hull, 3): Adhering hull, 4): Non-adhering hull.

green cotyledon] : [non-adhering/dark red cotyledon], suggesting that the traits are independent of each other.

4. Rutin concentration in dehulled grain in water

Rutin remained undegraded after the immersion of dehulled grain in water, but nearly 80% of rutin degraded 10 min after the addition of water to control flour (Fig. 2). This result suggests that the grain of 06T07-1-1g-0 had high activity of rutin-degrading enzyme[11], but the degradation of rutin was mostly avoidable in dehulled grain.

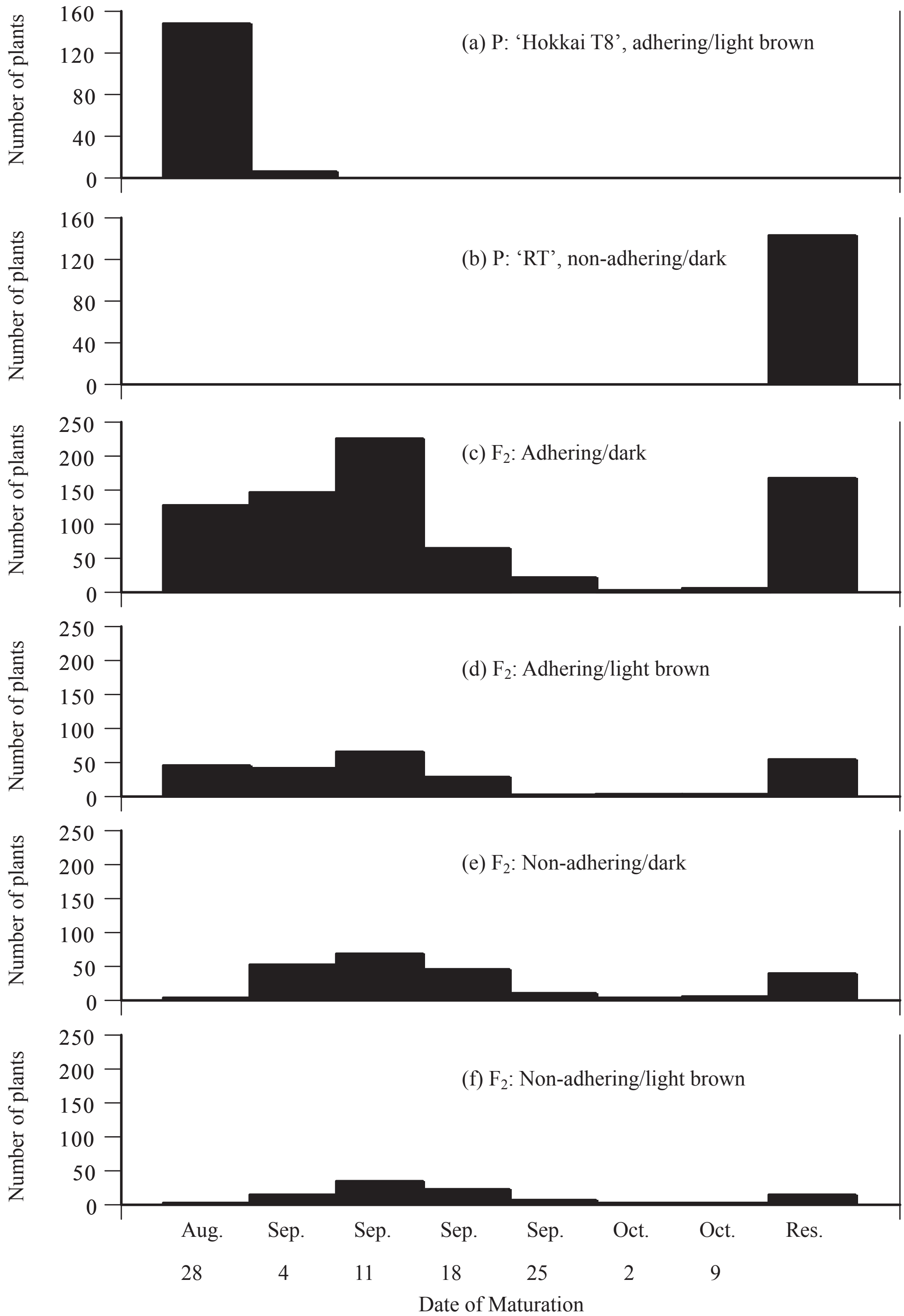

Fig. 1. Histograms of maturation time in the F_2 population of the cross between (a) 'Hokkai T8' and (b) 'RT'

The F_2 plants were categorized into 4 groups according to hull features: (c) adhering/dark, (d) adhering/light brown, (e) non-adhering/dark, (f) non-adhering/light brown. Res. means the residual plants killed by frost before maturation.

Discussion

Wang and Campbell[10] were the first to report the hybridization of rice-Tartary and Tartary buckwheats, but they pointed out hybridization was difficult, and backcrossing with Tartary buckwheat failed. In contrast, our hot-water emasculation method[5] made crossing rice-Tartary buckwheat ('RT') with Tartary buckwheat ('Hokkai T8') easy, and backcrossing with Tartary buckwheat had an average success rate of over 50% (Table 1). We consider that the difficulty reported by Wang and Campbell[10] was just technical, and was not due to a physiological cross-incompatibility. Our experience suggests that physical damage to the flower inhibits seed set or causes seed abortion, and hot-water emasculation can avoid the damage[5]. We believe that hybridization breeding of Tartary buckwheat is now feasible, as in other self-pollinating crops.

The distribution patterns of maturation time in the F_2 populations were roughly the same among the four hull-feature categories (Fig 1). This result suggests that non-adhering hull and earliness are independent, and early-maturing rice-Tartary type might be easily obtained by a single cross. The maturation time of several F_3 lines supported this idea (Table 3). However, F_2 segregates with a non-adhering hull matured a little later than those with an adhering hull in the first peak of the histograms (Fig. 1). We consider that this was caused by a difference in seed set, which was lower in the segregates with a non-adhering hull. This lower seed set might promote vegetative growth and delay maturation a little. However, rice-Tartary buckwheat is still a useful genetic resource for the trait of non-adhering hull because the trait is independent of extreme lateness.

We verified that a single recessive gene controls the trait of non-adhering hull[6,10] (Table 2). We had already revealed that the dark red cotyledon in 'Hokkai T10' is also controlled by a single recessive gene[7]. The segregation pattern fitted the 9:3:3:1 ratio of [adhering/green cotyledon] : [adhering/dark red cotyledon] : [non-adhering/green cotyledon] : [non-adhering/ dark red cotyledon] in the F_2 of rice-Tartary-like progeny × 'Hokkai T10', suggesting that the locus controlling non-adhering hull is not linked to that controlling dark red cotyledon. Selection of [non-adhering hull/dark red cotyledon]-type lines for seed sprout production appears feasible. However, the growth of F_2 segregates with dark red cotyledons was poor.

Because grain of rice-Tartary buckwheat can be dehulled easily, this may be an advantage for the use of Tartary buckwheat for rutin. Yasuda et al.[11] reported that rutin in Tartary buckwheat flour rapidly degraded when water was added because of the high activity of rutin-degrading enzyme. In this experiment, rapid degradation of rutin was also observed in the flour of rice-Tartary buckwheat after the addition of water, suggesting that the grain actually possessed high activity of rutin degradation. However, obvious degradation of rutin was not observed when the dehulled grain was immersed in water (Fig. 2). We speculated that this was caused by the structural isolation between rutin and rutin degrading enzyme in dehulled grain. Suzuki et al.[8] reported that the activity of flavonol-3-glucosidase (rutin degrading enzyme) was mainly observed in testa though rutin mainly existed in cotyledon. Thus, easily dehulled grain may allow the development of new high-rutin foods. We confirmed that more than 80% of the rutin remained after grain was boiled for 1 h (data not shown). However, the 1000 seed weight in all F_3 lines was still small (Table 3), and further crosses are needed to increase seed size.

In conclusion, rice-Tartary buckwheat promises to be a useful genetic resource for crossbreeding on account

Table 3. Characteristics of F_3 lines with non-adhering hull (in bulk)

Line	Date of maturation	Hull color	1000 seed (g)
Parents			
Rice-Tartary	not matured	D[1)]	9.96
Hokkai T8	Aug. 27	LB[2)]	19.23
Cross 'RT' × 'Hokkai T8'			
06T06-1-1g	Sept. 12	D	10.77
06T06-1-2g	Sept. 18	D	10.45
06T06-1-3g	not matured	LB	10.88
06T06-1-4g	Sept. 25	LB	12.46
06T06-1-5g	not matured	LB	10.53
06T06-1-6g	Sept.25	D and LB	11.32
06T06-1-7g	Sept. 12	D and LB	11.23
06T06-1-8g	Sept. 12	LB	11.82
06T06-1-9g	not matured	LB	10.56
06T06-1-10g	Sept. 18	D and LB	10.92
06T06-1-11g	Sept. 28	D and LB	9.14
Cross 'Hokkai T8' × 'RT'			
06T07-1-1g	Sept. 12	D	11.55
06T07-1-2g	Sept. 12	D and LB	11.94
06T07-1-3g	Sept. 12	D and LB	11.69
06T07-1-4g	not matured	D and LB	10.42

1): Dark hull, 2): Light brown hull.

Table 4. Segregation pattern of seed types (Tartary versus rice-Tartary) and cotyledon colors (green versus dark red) in F_2 plants (F_3 seeds)

Population		Number of plants				χ^2	P
	hull type	Adhering		Non-adhering		for	
	cotyledon color	G[1)]	DR[2)]	G	DR	9:3:3:1	
06T07-1-1g-1g × 'Hokkai T10'							
07T11-1		35	8	6	1	4.54	0.21
07T11-2		32	9	8	0	3.93	0.27
07T11-3		29	6	12	1	3.48	0.32
07T11-4		29	10	8	1	1.70	0.64
07T11-5		30	7	3	3	4.75	0.19
07T11-6		28	8	11	1	1.93	0.59
07T11-7		24	8	15	4	4.32	0.23
07T11-8		19	17	8	4	9.93	0.02
Total		226	73	71	15	3.85	0.28
06T07-1-1g-2g × 'Hokkai T10'							
07T12-1		26	10	11	2	0.89	0.83
07T12-2		34	7	5	1	5.46	0.14
07T12-3		30	11	9	1	1.81	0.61
07T12-4		29	8	10	2	0.67	0.88
Total		119	36	35	6	3.98	0.26

1): Green cotyledon, 2): Dark red cotyledon

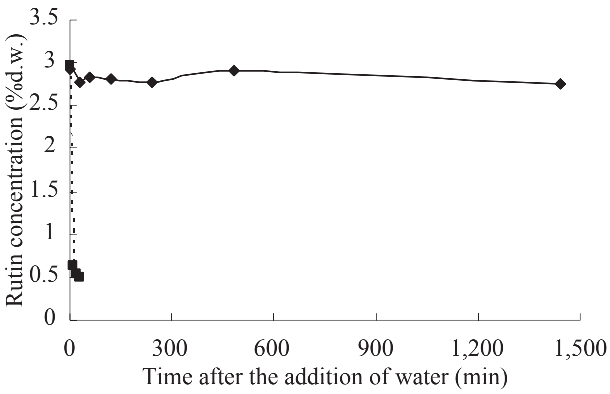

Fig. 2. Change in rutin concentration after the addition of water to dehulled whole grain or flour of rice-Tartary buckwheat (line 06X07-1-1g-0)
—◆—: Dehulled whole grain, -■-: Flour

of its crossability, the independence between non-adhering hull and extreme lateness, and the independence between non-adhering hull and dark red cotyledon. The inheritance of non-adhering hull is simple, and the phenotype is controlled by a single recessive gene. Such a trait will facilitate grain use and efficient intake of rutin.

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