

Supplementary Table S1: List of Primers Used for Sequencing.

#	Direction	Oligonucleotide sequence
1	Forward	5' GCAAAGATGAACAGAGGTGG 3'
2	Forward	5' GAGGCATATTTAACGTTATAGCAGGGC 3'
3	Forward	5' GGCTTAGTTCCATCTAAGAGATG 3'
4	Forward	5' CAAGAGCCATGCGAGGTAGAG 3'
*5	Forward	5' GGCATGTATTTTGGTGAAGTCG 3'
6	Forward	5' CAAGCTGCGATCTTTAGGCTG 3'
7	Forward	5' GCCAAGAGATCAGATGTGCAG 3'
8	Forward	5' GTGTCCACTGGCCAGATAGTATC 3'
9	Forward	5' GCACCAACCGCATATACAGG 3'
10	Reverse	5' GGATTCTGATGCTTGATTAATTCC 3'
11	Reverse	5' CTGGTCTGCCTAAAGATCAATTG 3'
*12	Reverse	5' GTTGTCGTAGTACGAATTGTACCCGAC 3'
13	Reverse	5' GCTTGCACCACATGGACAAGTCTATCG 3'

* Primers used for genotype screening of *hd1*.

Supplementary Table S2. Agricultural Traits of Rice NILs Grown in 2020.

Yield and yield components	<i>ss2a Hd1</i>	<i>ss2a Hd1 hd1</i>	<i>ss2a hd1</i>
Days to heading (days)	112	94	72
Days to maturation (days)	48	44	40
Plant height (cm)	110 ± 0.4a	102 ± 0.2b	98 ± 0.6c
Culm length (cm)	88 ± 0.7a	81 ± 0.2b	77 ± 0.5c
Ear length (cm)	22 ± 0.4a	21 ± 0.1a	21 ± 0.2a
Ear number (No.)	21 ± 1.4a	22 ± 0.3a	21 ± 1.6a
Whole plant dry weight (g)	128.5 ± 11.5a	107.4 ± 1.8ab	83.9 ± 6.1b
Dry straw weight (g)	72.8 ± 6.5a	53.6 ± 1.0ab	37.6 ± 2.2b
Total grain weight (g)	53.9 ± 4.9a	48.5 ± 0.8a	43.7 ± 3.8a
Number of grains per 10 g (No.)	365.3 ± 4.4b	354.7 ± 0.6b	417.9 ± 7.0a
Thousand grain weight (g)	27.4 ± 0.3a	28.2 ± 0.1a	24.0 ± 0.4b
Percentages of fertile seeds (%)	82.2 ± 1.6	91.0 ± 0.2	87.7 ± 2.1
Thousand dehulled-grain weight (g)	28.9 ± 0.2a	27.0 ± 0.0b	26.4 ± 0.3b
Percentages of green immature grains (%)	4.6 ± 1.0a	4.0 ± 0.8a	0.1 ± 0.0b

Average values are presented as mean ± standard error. n = 9, 20, and 8 for *ss2a Hd1*, *ss2a Hd1 hd1*, and *ss2a hd1*, respectively. Different letters (a-c) indicate significantly different from one another as determined by Tukey-Kramer method ($p < 0.05$).

Supplementary Table S3. Agricultural Traits of Rice NILs Grown in 2021.

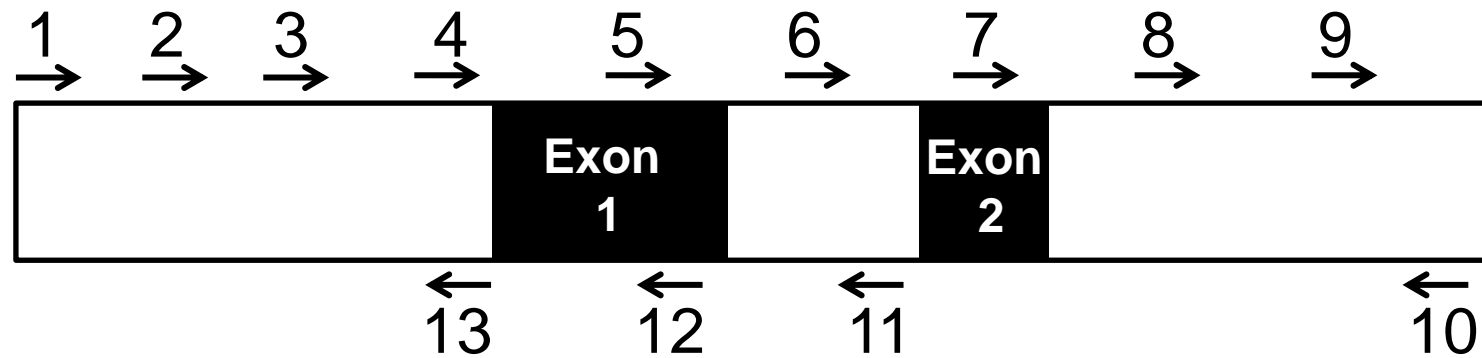
Yield and yield components	<i>ss2a Hd1</i>	<i>ss2a Hd1 hd1</i>	<i>ss2a hd1</i>
Days to heading (days)	104	92	78
Days to maturation (days)	54	47	40
Plant height (cm)	117.2 ± 0.5a	112.5 ± 0.6b	106.2 ± 0.6c
Culm length (cm)	95.3 ± 0.5a	90.2 ± 0.9b	84.0 ± 0.7c
Ear length (cm)	21.9 ± 0.3a	22.3 ± 0.4a	22.2 ± 0.4a
Ear number (No.)	20.1 ± 0.6a	21.6 ± 1.3a	21.6 ± 1.1a
Whole plant dry weight (g)	106.0 ± 6.2a	99.0 ± 6.6a	77.1 ± 4.9b
Dry straw weight (g)	65.9 ± 3.9a	46.7 ± 3.2b	34.4 ± 1.7c
Total grain weight (g)	34.1 ± 2.3b	47.5 ± 3.2a	37.7 ± 3.2b
Number of grains per 10 g (No.)	427.1 ± 8.9a	347.6 ± 2.4c	362.9 ± 5.5b
Thousand grain weight (g)	23.6 ± 0.5b	28.8 ± 0.2a	27.7 ± 0.4a
Percentages of fertile seeds (%)	78.7 ± 2.0b	89.8 ± 0.7a	82.7 ± 1.2b
Thousand dehulled-grain weight (g)	29.8 ± 0.2c	32.1 ± 0.2b	33.4 ± 0.3a
Percentages of green immature grains (%)	43.2 ± 2.5a	12.2 ± 1.1b	4.4 ± 0.7c

Average values are presented as mean ± standard error (n=20). Different letters (a-c) indicate significantly different from one another as determined by Tukey-Kramer method ($p < 0.05$).

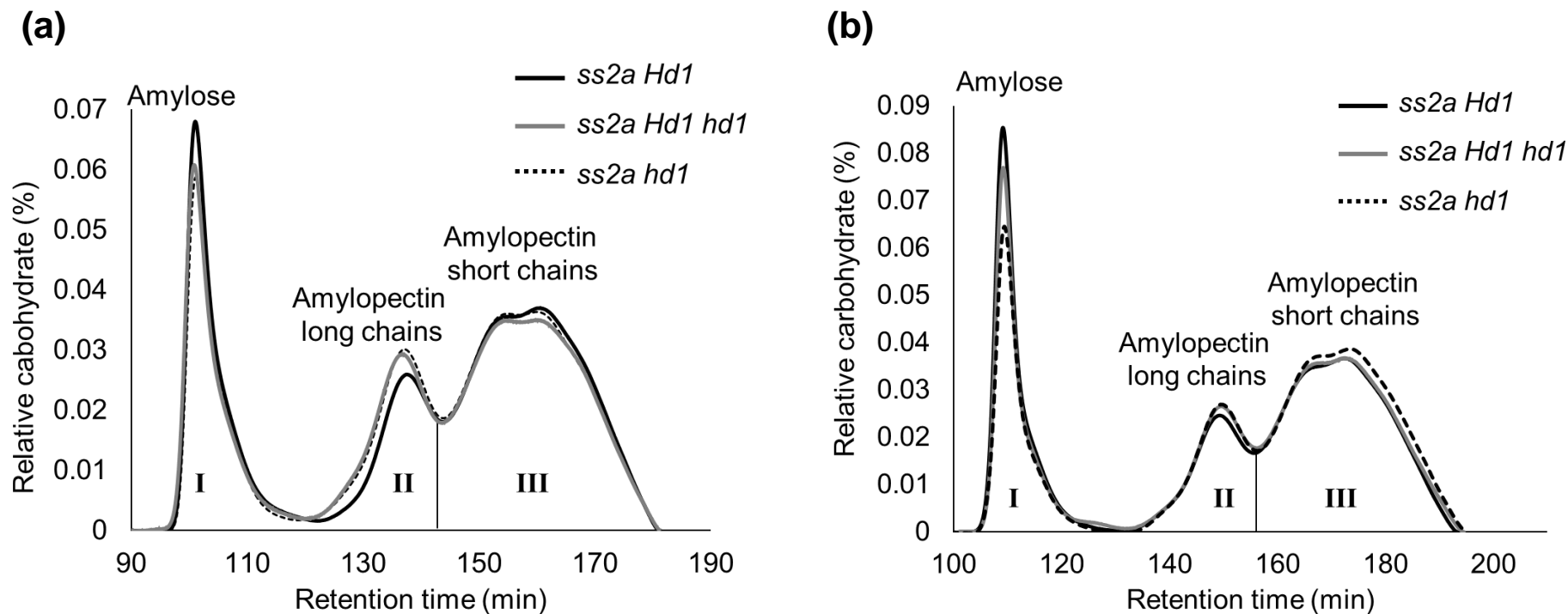
Supplementary Table S4. Average of daily temperature during the seed development.

Line	Maximum		Minimum		Day average	
	2020	2021	2020	2021	2020	2021
<i>ss2a Hd1</i>	21.4	21.7	13.4	13.1	17.0	17.0
<i>ss2a Hd1 hd1</i>	27.5	25.5	19.4	16.7	22.8	20.8
<i>ss2a hd1</i>	30.8	28.5	23.1	20.9	26.6	24.5

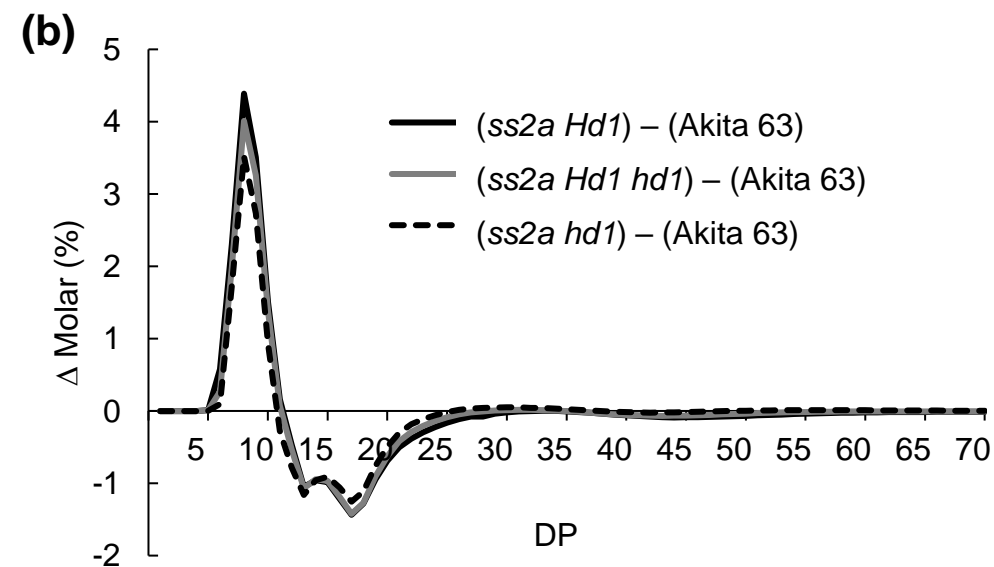
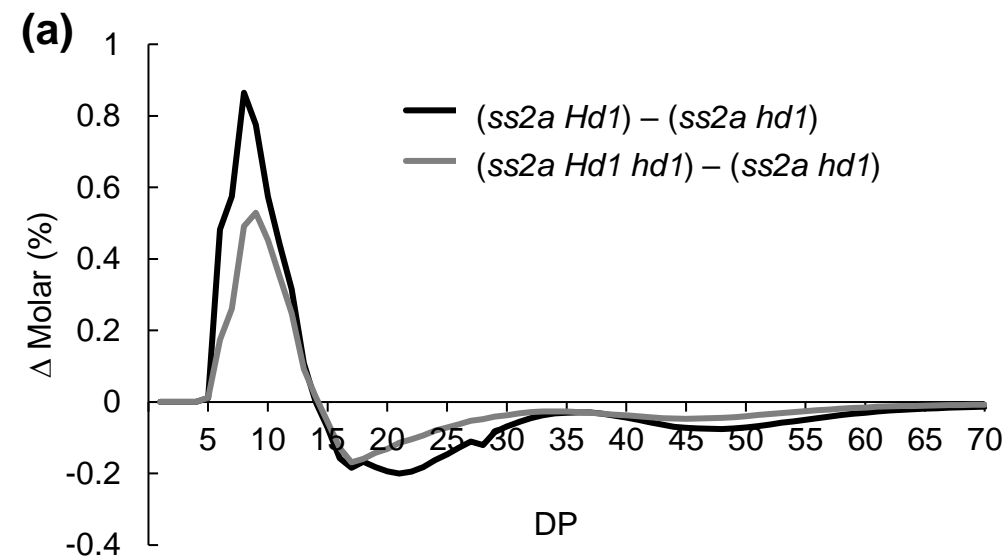
Meteoroidal dataset of the rice field was obtained from the Japan Meteorological Agency. Average temperature of daily high, low, and average were calculated for 2020 and 2021.



Supplementary Figure S1. Locations of primers used for sequencing the *Hd1* gene of rice accessions



Supplementary Figure S2. Elution profiles of rice accessions by gel filtration chromatography of debranched starch prepared from rice seeds harvested in **(a)** 2020 and **(b)** 2021. Three fractions (I, II, and III) were divided at the troughs of carbohydrate-content curve. Amylose elutes in fraction I, long chains of amylopectin branches elute in fraction II, and short chains of amylopectin branches elute in fraction III. Elution profiles are one of the representatives of at least three experiments. Exact values are shown in Table 4 and supplementary table 4.



Supplementary Figure S3. Differences in amylopectin branch structure among rice NILs. Samples were prepared using rice seeds harvested in 2020. **(a)** The effects of the *Hd1* genotypes on amylopectin branch structure shown by subtraction curves as indicated. **(b)** The effect of loss of SSIIa on amylopectin structure shown by subtraction curves as indicated. Each panel shows one typical representative data set of at least three replications.

(a)	Akita63	MNYNFGGNVFDQEVGVGGE GGGGGEGSGCPWARPCDGCRAAPSVVYCRADAAYLCASDA	60
	HS66	MNYNFGGNVFDQEVGVGGE GGGGGEGSGCPWARPCDGCRAAPSVVYCRADAAYLCASDA	60
	Sasanishiki	MNYNFGGNVFDQEVGVGGE GGGGGEGSGCPWARPCDGCRAAPSVVYCRADAAYLCASDA	60

	Akita63	RVHAANRVASRHERVRVCEACERAPAALACRADAAALCVACDVQVYSANPLARRHQRPV	120
	HS66	RVHAANRVASRHERVRVCEACERAPAALACRADAAALCVACDVQVYSANPLARRHQRPV	120
	Sasanishiki	RVHAANRVASRHERVRVCEACERAPAALACRADAAALCVACDVQVYSANPLARRHQRPV	120

	Akita63	APLPAITIPATSVLAEAVVATATVLGDKDEEVDSWLLLSKSDNNNNNNNNNDNDNDNDN	180
	HS66	APLPAITIPATSVLAEAVVATATVLGDKDEEVDSWLLLSKSDNNNNNNNNNDNDNDNDN	180
	Sasanishiki	APLPAITIPATSVLAEAVVATATVLGDKDEEVDSWLLLSKSDNNNNNNNNNDNDNDNDN	180

	Akita63	NSNSSNNGMYFGEVDEYFDLVGYNSYYDNRIENNQDRQYGMHEQQEQEQEQEQEQEMQKEFAE	240
	HS66	NSNSSNNGMYFGEVDEYFDLVGYNSYYDNRIENNQDRQYGMHEQQEQEQEQEQEQEMQKEFAE	240
	Sasanishiki	NSNSSNNGMYFGEVDEYFDLVGYNSYYDNRIENNQDRQYGMHEQQEQEQEQEQEQEMQKEFAE	240

	Akita63	KEGSECVVMELWEQTRPPP	259
	HS66	KEGSECVVMELWEQTRPPP	259
	Sasanishiki	KEGSECVVMELWEQTRPPP	259

Supplementary Figure S4. (a) Alignment of Hd1 in Akita 63, HS66, and Sasanishiki. Asterisks indicate identical amino acid sequences.

Akita63	MNYNFGGNVFDQEVGVGGE GGGGGEGSGCPWARPCDGCRAAPSVVYCRADAAYLCASCD	60
Kasalath	MNYNFGGNVFDQEVGVGGE GGGGGEGSGCPWARPCDGCRAAPSVVYCRADAAYLCASCD	60
Ginbouzu	MNYNFGGNVFDQEVGVGGE GGGGGEGSGCPWARPCDGCRAAPSVVYCRADAAYLCASCD	60
Koshihikari	MNYNFGGNVFDQEVGVGGE GGGGGEGSGCPWARPCDGCRAAPSVVYCRADAAYLCASCD	60

Akita63	RVHAANRVASRHERVRVCEACERAPAAALACRADAAAALCVACDVQVYSANPLARRHQRPV	120
Kasalath	RVHAANRVASRHERVRVCEACEQAPAAALACRADAAAALCVACDVQVHSANPLARRHQRPV	120
Ginbouzu	RVHAANRVASRHERVRVCEACERAPAAALACRADAAAALCVACDVQVYSANPLARRHQRPV	120
Koshihikari	RVHAANRVASRHERVRVCEACERAPAAALACRADAAAALCVACDVQVHSAN-----	109
*****:*****:***		
Akita63	APLPAITIPATSVLAEAVVATATVLGDKDEEVDSWLLLLSKDSDNNNNNNNNNDNDNDNDNN	180
Kasalath	APLPAITIPATSVLAEAVVATATVLGGKDEEVDSWIIILSKDSDNNNNN-----NN	169
Ginbouzu	APLPAITIPATSVLAEAVVATATVLGDKDEEVDSWLLLLSKDSDNNNNNNNNNDNDNDNDNN	180
Koshihikari	-PLPAITIPATSVLAEAVVATATVLGDKDEEVDSWLLLLSKDSDNNNNNNNNNDNDNDNDNN	168
*****.*****.:*****:*** **		
Akita63	NSNSSNNGMYFGEVDEYFDLVGYNSYYDNRIENNQDRQYGMHEQQEQQQQQEQMKEFAE	240
Kasalath	NSNSSNNGMYFGEVDEYFDLVGYNSYYDNRIENNQDQQYGMHEQQEQQQQQEQMKEFAE	229
Ginbouzu	NSNSSNNGMYFGEVDEYFDLVGYNSYYDNRIENNQDRQYGMHEQQEQQQQQEQMKEFAE	240
Koshihikari	NSNSSNNGMYFGEVDEYFDLVGYNSYYDNRIENNQDRQYGMHEQQEQQQQQEQMKEFAE	228
*****:*****		
Akita63	KEGSECVVMELWEQTRPPP-----	259
Kasalath	KEGSECVVPSQITMLSEQQHSYG VVGADQAASMTAGVSAYTDSISNSISLINGGGYSTR	289
Ginbouzu	KEGSECVVPSQITMLSEQQHSYG VVGADQAASMTAGVSAYTDSISNSISFSSMEAGIVP	300
Koshihikari	KEGSECVVPSQITMLSEQQHSYG VVGADQAASMTAGVSAYTDSISNSISFSSMEAGIVP	288
***** .		
Akita63	-----	259
Kasalath	QHG--D-RYAKFQHPD---TCWSNQSLRLSLA-----SDVPSLQLHGQGG-----	328
Ginbouzu	DSTVIDMPNSRILTPAGAINLFSGPSLQMSLHFSSMDREARVLRREKKKARKFEKTIRY	360
Koshihikari	DSTVIDMPNSRILTPAGAINLFSGPSLQMSLHFSSMDREARVLRREKKKARKFEKTIRY	348
Akita63	-----	259
Kasalath	-----QGAQVQGEEEE-----GQEV-----	342
Ginbouzu	ETRKAYAEARPRIKGRFAKRSDVQIEVDQMFSTAALSDGSYGTVPWF	407
Koshihikari	ETRKAYAEARPRIKGRFAKRSDVQIEVDQMFSTAALSDGSYGTVPWF	395

Supplementary Figure S4. (b) Alignment of Hd1 in Akita 63, Kasalath, Ginbouzu, and Koshihikari. Asterisks, dashes, periods, and colons indicate identical amino acid sequences, missing residues, semi-conservative substitutions, conservative substitutions, respectively.

Ginbouzu	MNYNFGGNVFDQEVGVGEGGGGGEGSGCPWARPCDGCRAAPSVVYCRADAAYLCASDA	60
Hoshinoyume	MNYNFGGNVFDQEVGVGEGGGGGEGSGCPWARPCDGCRAAPSVVYCRADAAYLCASDA	60
Hayamasari	MNYNFGGNVFDQEVGVGEGGGGGEGSGCPWARPCDGCRAAPSVVYCRADAAYLCASDA	60

Ginbouzu	RVHAANRVASRHERVRVCEACERAPAALACRADAAALCVACDVQVYSANPLARRHQRPV	120
Hoshinoyume	RVHAANRVASRHERVRVCEACERAPAALACRADAAALCVACDVQVYSANPLARRHQRPV	120
Hayamasari	RVHAANRVASRHERVRVCEACERAPAALACRADAAALCVACDVQVYSANPLARRHQRPV	120

Ginbouzu	APLPAITIPATSVLAEAVVATATVLGDKDEEVDSWLLLSKSDSDNNNNNNNNNDNDNDNDNN	180
Hoshinoyume	APLPAITIPATSVLAEAVVATATVLGDKDEEVDSWLLLSKSDSDNNNNNNNNNDNDNDNDNN	180
Hayamasari	APLPAITIPATSVLAEAVVATATVLGDKDEEVDSWLLLSKSDSDNNNNNNNNNDNDNDNDNN	180

Ginbouzu	NSNSSNNGMYFGEVDEYFDLVGYNSYYDNRIENNQDRQYGMHEQQEQQQQQEMQKEFAE	240
Hoshinoyume	NSNSSNNGMYFGEVDEYFDLVGYNSYYDNRIENNQDRQYGMHEQQEQQQQQEMQKEFAE	240
Hayamasari	NSNSSNNGMYFGEVDEYFDLVGYNSYYDNRIENNQDRQYGMHEQQEQQQQQEMQKEFAE	240

Ginbouzu	KEGSECVVPSQITMLSEQQHSGYGVGADQAASMTAGVSAYTDSISNSISFSSMEAGIVP	300
Hoshinoyume	KEGSECVVPSQITMLSEQQHSGYGVGADQAASMTAGVSAYTDSISNSISFSSMEAGIVP	300
Hayamasari	KEGSECVVPSQITMLSEQQHSGYGVGADQAASMTAGVSAYTDSISNSISFSSMEAGIVP	300

Ginbouzu	DSTVIDMPNSRILTPAGAINLFSGPSLQMSLHFSSMDREARVRLRYREKKKARKFEKTIRY	360
Hoshinoyume	DSTVIDMPNSRILTPAGAINLFSGPSLQMSLHFSSMDREARVRLRYREKKKARKFEKTIRY	360
Hayamasari	DSTVIDMPNSRILTPAGAINLFSGPSLQMSLHFSSMDREARVRLRYREKKKARKFEKTIRY	360

Ginbouzu	ETRKAYAEARPRIKGRFAKRSDVQIEVDQMFSTAALSDGSYGTVPWF	407
Hoshinoyume	ETRKAYAEARPRIKGRFAKRSDVQIEVDQMFSTAALSDGSYGTVPWF	407
Hayamasari	ETRKAYAEARPRIKGRFAKRSDVQIEVDQMFSTAALSDGSYGTVPWF	407

Supplementary Figure S4. (c) Alignment of Hd1 in Ginbouzu, Hoshinoyume, and Hayamasari. Asterisks indicate identical amino acid sequences.