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Psy-E1, located at the same locus as Psy-A1, contributes strong yellowness to durum wheat but may cause yield loss in Japan.

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Abstract

Strong yellow color, caused by carotenoid accumulation, in semolina flour made from durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.)) is one of the most important phenotypes for pasta production. The first step in the carotenoid biosynthesis pathway, which is catalyzed by phytoene synthase (PSY), is a bottleneck, and allelic variation of *Psy-A1* in durum wheat produces different yellow pigment contents (YPC) in seeds. Durum wheat carrying leaf rust resistance gene *LR19*, which was translocated from wheat relative *Thinopyrum ponticum* chromosome 7E to durum wheat chromosome 7A, is known to produce high YPC, and the causal gene is presumed to be *Psy-E1*, which is tightly linked to *LR19*. In this study, *Psy-E1* produced more YPC than the original *Psy-A1* alleles in durum wheat, and YPC production decreased in the order of *Psy-E1* > *Psy-A1o* > *Psy-A1l* > *Psy-A1k*. Segregation analysis demonstrated that *Psy-E1* is located at the *Psy-A1* locus on chromosome 7A. In a 2-year field test of near-isogenic materials, *Psy-E1* was accompanied by yield loss with decreases in grain number per spike, volume weight and thousand-kernel weight under moisture conditions typical of wheat-growing areas of Japan. Thus, *Psy-E1* has the potential to contribute high YPC in durum wheat breeding programs, although the applicable cultivation environments are limited.

Introduction

For pasta products made from semolina flour of durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.)), yellowness and elastic texture with high protein contents and gluten strength are desirable traits. In particular, a bright yellow color, which is caused by carotenoid yellow pigment content (YPC), is preferred for pasta products (Hentschel et al. 2002; Lachman et al. 2017). In addition to their role of a visual quality of pasta products, carotenoids have important roles in human nutrition and health owing to their provitamin A activity and antioxidant properties (Ficco et al. 2014; Abdel-Aal et al. 2007).

YPC of wheat is a stable phenotype influenced by genetics and showing high heritability (Clarke et al. 2006; Digesù et al. 2009). Recently, many genetic studies have examined carotenoid biosynthesis in plants. *Triticum* species, including durum wheat, accumulate yellow pigment in the endosperm; this pigment is a mixture of carotenoids, mainly lutein (Prins et al. 1997; Digesù et al. 2009). Lutein is biosynthesized from geranylgeranyl diphosphate, which is converted to phytoene by phytoene synthase (PSY) as the initial step, followed by the downstream reactions of the terpenoid biosynthesis pathway (Ramachandran et al. 2010). Phytoene is converted to lycopene in a desaturation step catalyzed by phytoene desaturase (Pds) (Bartley et al. 1999) and zeta-carotene desaturase (Zds) (Albrecht et al. 1995) and an isomerization step catalyzed by carotenoid isomerase (CRTiso) (Matthews 2003). Lycopene is then converted to lutein through cyclization by lycopene-β-cyclase (βLCY) and lycopene-ε-cyclase (εLCY) and hydroxylation by carotenoid β hydroxylase (βOHase) and carotenoid □ hydroxylase (□OHase) (Fraser and Bramley 2004). In this pathway, three bottlenecks are known: phytoene synthesis by PSY, lycopene cyclization and carotene hydroxylation (Cong et al. 2009; Lindgren et al. 2003; Zhou et al. 2022).

There are three PSY paralogs in wheat: PSY1 in leaf, which is involved in carotenoid accumulation; PSY2 in green tissues, which is involved in photosynthesis; and PSY3 in root, which regulates ABA biosynthesis under abiotic stress (Dibari et al. 2012; Li et al. 2008; Pozniak et al. 2007; Zhang and Dubcovsky 2008; Zhou et al. 2022). Zhang and Dubcovsky (2008) also reported two QTLs related to yellow pigment accumulation, *Psy-A1* and *Psy-B1*, on chromosome (Chr.) group 7 in durum wheat. Allelic variations in *PSY1* genes were later found in durum wheat and compered their effect for YPC, the effect order the YPC from high to low was *Psy-A1o*, *Psy-A1l* and *Psy-A1a* by recombination inbred lines (Singh et al. 2009). Other studies found that, under some conditions, *Psy-A1o* and *Psy-A1l* had the same effect on YPC, both stronger than *Psy-A1a*, that *Psy-B1a* and *Psy-B1b* had the same effect (Campos et al. 2016), and that *Psy-B1f* had a stronger effect than *Psy-B1g* (He et al. 2009).

Wild relatives of wheat, especially *Thinopyrum ponticum* (syn: *Th. elongatum*, *Agropyron elongatum* and *Lophopyrum elongatum*), have been shown to be a resource for leaf rust resistance in breeding programs (McCallum et al. 2016; Prasad et al. 2020). One leaf rust resistance gene, *Lr19*, has been transferred from *A. elongatum* (*Th. ponticum*) on Chr.7E to the long arm of wheat Chr. 7D. A translocated segment containing *Lr19* was associated with higher biomass and grain yield under irrigated conditions and with yellow endosperm designated causal gene as *Y* (Monneveux et al. 2003; Singh et al. 1998). Zhang et al. (2005) and Gennaro et al. (2009) generated durum wheat–*Th. ponticum* recombinant lines in which a segment containing *Lr19* with *Y* was translocated to Chr. 7A in durum wheat.

Y gene derived from *Th. ponticum* was presumed to be *Psy-E1*, the orthologue of *Psy-1*, and *Psy-E1* is involved in YPC (Zhang and Dubcovsky 2008). The genetic distance between *Lr19* and *Psy-E1* was estimated to be 9.8 cM (Xu et al. 2023). However, *Lr19*-linked *Psy-E1* has been not used for durum wheat breeding, and its effects on yellow endosperm in durum wheat are unclear. Our objective in this study was to evaluate the use of *Psy-E1* in durum wheat breeding. We evaluated the degree of yellowness in *Psy-E1* endosperm and its effect on agronomic traits compared with the original *Psy-A1* alleles in durum wheat. We also tested whether it would be possible to pyramid *Psy-E1* with *Psy-A1* to increase yellowness in endosperm.

Materials and methods

DNA sequencing of Psy-A1 gene in durum cultivar 'Setodure'

DNA was extracted by the potassium acetate method (Dellaporta et al. 1983). DNA templates were amplified in a PCR Thermal Cycler Dice (TaKaRa Bio, Shiga, Japan) with LA Taq and 10× LA PCR Buffer II (TaKaRa Bio) and primers listed in Table S1. PCR amplification of the open reading frame of *Psy-A1* was an initial 94°C for 1 min, followed by 35 cycles of 95°C for 10 s and 72°C for 10 min. The amplified PCR fragments were separated by agarose gel electrophoresis, visualized with Gel Red stain (Biotium, Fremont, CA, USA) and extracted with a Qiaquick Gel Extraction Kit (Qiagen, Hilden, Germany). Cycle sequencing reactions were performed in a PCR Thermal Cycler Dice with BigDye Terminator v. 3.1 with sequencing primers listed in Table S1 and purified with BigDye Terminator (Applied Biosystems,

Waltham, MA, USA) following the manufacturer's instructions. DNA was sequenced on a 3130xl Genetic Analyzer (Applied Biosystems).

Plant materials and cultivation conditions.

To develop near-isogenic lines (NILs), we used the durum wheat cultivar 'Setodure' (possessing *Psy-A1k*; see Results) as the recurrent parent. Durum wheat cultivars 'AC Navigator' and 'Enterprise', and experimental line Ap1-22, which possess *Psy-A1l*, *Psy-A1o* and *Lr19*, respectively, were used as donors. By backcrossing each of the donors to 'Setodure', we developed BC_6F_2 plants containing each gene of interest by DNA marker-assisted selection.

The three NILs and 'Setodure' were grown in a research field at the Western Region Agricultural Research Center (WARC), Fukuyama, Hiroshima, Japan (lat. 34°30'4"N, lng. 133°23'12"E). materials 756 seeds were sown in a strip 0.7 m wide × 7.2 m long in November and harvested in June. There were 4 replicates of materials in two seasons, 2020–21 and 2021–22 (later referred to as harvest years 2021 and 2022, respectively).

Psy-A1, Psy-E1 and Lr19 genotyping

DNA was extracted as described above. All PCR reactions used Thermal Cycler Dice with Quick Taq HS (Toyobo, Osaka, Japan), and the amplified PCR fragments were separated and visualized as above. PCR amplification of *Psy-A1* alleles (*Psy-A1k*, *Psy-A1l* and *Psy-A1o*) was an initial 95°C for 2 min, followed by 35 cycles of 94°C for 30 s, 65°C for 30 s, and 68°C for 2 min. Amplification of *Psy-E1* was an initial 95°C for 2 min; 10 cycles with a touchdown step of 94°C for 30 s, 65–56°C for 30 s with a decrease of 1°C per cycle, and 68°C for 1.5 min; and then 25 cycles of 94°C for 30 s, 55°C for 30 s and 68°C for 1.5 min. Amplification of *Lr19* was an initial 95°C for 2 min; 8 cycles with a touchdown step of 94°C for 30 s, 68–61°C for 30 s with a decrease of 1°C per cycle, and 68°C for 1 min; and then 22 cycles of 94°C for 30 s, 60°C for 30 s and 68°C for 1 min. The multiplex PCR of *Psy-A1* and *Psy-E1* with HotStarTaq *Plus* (Qiagen) was an initial 95°C for 5 min, followed by 35 cycles of 94°C for 1 min, 65°C for 1 min, and 72°C for 1 min. *Lr19* with *Waxy-B1* as an internal control was amplified as for genotyping of *Lr19* alone. Primers listed in Table S1

Segregation analysis

Segregation ratios of *Psy-A1* and *Psy-E1* were calculated in three F_2 populations: *Lr19* NIL × 'Setodure' (*Psy-A1k*), *Lr19* NIL × *Psy-A1l* NIL and *Lr19* NIL × *Psy-A1o* NIL. Genotyping was performed by multiplex PCR of *Psy-A1* and *Psy-E1.*

Whole grain, straight flour and semolina flour production

To measure whole-grain production, 30 g of dried seeds were pulverized in a cyclone mill (Cyclotec 1093; Foss, Hillerød, Denmark) with a 0.5-mm sieve.

To measure straight flour production by small-scale milling, 150 g of dried seeds were milled in a Quadrumat Jr. (C.W. Brabender Instruments, Inc., South Hackensack, NJ, USA) without a sieve. The milled samples were sieved in a rotary sifter with a 355-μm sieve for 5 min at 360 rpm, and the material that passed through the sieve was used as straight flour.

To measure semolina flour production by large-scale milling, seed from 4 replicates was combined, and 2 kg of seeds were milled in a Bühler Laboratory Mill (Bühler, Uzwil, Switzerland) following Cereal & Grains Association method AACC 26-41.01 (AACC Approved Methods of Analysis, 2009b).

Pasta production

Pasta processing using semolina flour in 2021 and the boiling test were performed following AACC 66-42.01 (AACC Approved Methods of Analysis, 2009c) and AACC 66-50.01 (AACC Approved Methods of Analysis, 2009d), respectively.

Yellow pigment content (YPC) quantification and b* value of CIELAB measurement

YPC was extracted from whole grain, straight flour, and semolina flour by AACC 14-50.01 (AACC Approved Methods of Analysis, 2009a) and quantified on a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The b* value of the CIELAB color space, indicative of yellow color intensity, was measured on a CM3500d spectrophotometer (Konica Minolta, Inc., Tokyo, Japan) with light source C and a 2° field for whole grain, straight flour, and semolina flour, and spectrophotometer CM-5 (Konica Minolta) with light source C and a 2° field for dried and boiled spaghetti. The dried spaghetti pieces were lined up without gaps and measured. Spaghetti that had been boiled for 5 min was packed in a transparent plastic bag and measured the next day.

Cultivation test

Agronomic traits were assessed in 2020 and 2021. Heading date and maturity date were determined as the accumulated days after sowing, and culm length was measured by our routine laboratory protocol following the International Union for the Protection of New Varieties of Plants (UPOV) guidelines (https://www.upov.int/edocs/tgdocs/en/tg003.pdf). Yield and spike number were measured by our routine laboratory protocols: Spikes and grains were harvested from each plot in 2020 and 2021. Awn and spike length were measured with a ruler, and spikelets and grains per spike were counted manually. Spikelet density was based on spikelet number and spike length. Grain protein content and volume weight were analyzed with a near-infrared spectrophotometer (IM9500; Perten Instruments, Stockholm, Sweden). Grain diameter, thousand-kernel weight and grain hardness were measured with a single-kernel characterization system (SKCS4100; Perten Instruments). Samples were collected from 4 replicates except for spike number, culm length, and spike traits (spike length, awn length, spikelet number, spikelet density and grain number). Spike number was averaged from two areas within each of the 4 replicates. Culm length was averaged from 10 culms in each of the 4 replicates. The spike traits were averaged from 10 spikes in each of the 4 replicates.

Statistical analysis

The YPC and b* values of ‘Setodure’ and NILs were analyzed by ANOVA and Tukey’s HSD at $P < 0.05$. For segregation analysis of *Psy-E1* and *Psy-A1*, chi-squared analysis was applied with two models. By the same-locus model, the expected segregation ratio for homozygous *Psy-E1* : heterozygous *Psy-E1*/*Psy-A1* : homozygous *Psy-A1* was 1:2:1. By the different-locus model, the expected segregation ratio for was both of them : only *Psy-E1* : only *Psy-A1* : null of them as 9:3:3:1. All statistics were analyzed in R version 4.2.0 software (R Core Team 2023).

Results

Genotype determination

When the genotype of *Psy-A1* in ‘Setodure’ was examined by DNA sequencing with primer walking, it was a complete match to *Psy-A1k* (FJ393522) in *T. turgidum* subsp. *dicoccoides* and to FJ393527 in *T. aestivum* subsp. *Spelta*. Thus, the sequence in ’Setodure’ is classified as *Psy-A1k* (registered as LC778135).

The *Psy1* genotypes of ‘Setodure’ (*Psy-A1k*), *Psy-A1l* NIL and *Psy-A1o* NIL were clearly distinguished by a codominant DNA marker for *Psy-A1* alleles (Fig. 1a). *Lr19* NIL was clearly distinguished by a dominant DNA marker for *Lr19* (Fig. 1d). In *Lr19* NIL, *Psy-E1*, but not *Psy-A1*, could be amplified with a novel *Psy-E1* gene-specific DNA marker (referred to as EU096095 in *Th. ponticum*) under both simplex and multiplex PCR conditions (Fig. 1b, c).

YPC quantification and b* value of CIELAB measurement

To assess the effect of *Psy* genotype on yellowness of flour and pasta, we measured YPC and the b* value of the CIELAB color space in ‘Setodure’ and the three NILs. YPC and b* were the highest in *Lr19* NIL and lowest in ‘Setodure’ (Figs. 2, 3). For both YPC and b*, the genotype effects in order from high to low were *Lr19* NIL > *Psy-A1o* NIL > *Psy-A1l* NIL > ‘Setodure’ (*Psy-A1k*), and there were significant differences among the genotypes regardless of sample type (whole grain or straight flour) and for both single years and 2-year averages (Figs. 2a, b; 3a, b).

YPC and b* in semolina flour in 2021, 2022 and the 2-year average (Figs. 2c, 3c) and b* in dry and boiled spaghetti in 2021 (Fig. 3d, e) were obtained without replication because large amounts of seed were needed for large-scale milling to obtain semolina flour for making pasta. The orders of the four genotypes were the same for YPC and b* in semolina flour and for b* in dry and boiled spaghetti as those for whole grain and straight flour.

Segregation analysis

To test our hypothesis that *Psy-E1* lies at the same locus as *Psy-A1*, we analyzed segregation ratios in three F_2 populations produced by crossing *Lr19* NIL with ‘Setodure’ (*Psy-A1k*), *Psy-A1l* NIL or *Psy-A1o*

NIL by chi-squared analysis with same- and different-locus models. *Psy-A1*- and/or *Psy-E1*-specific fragments were amplified in all F_2 individuals (Table 1, 2), none of any F_2 individuals amplified neither *Psy-A1*- nor *Psy-E1*-specific fragments (Table 2). In three F_2 populations produced by crossing *Lr19* NIL with 'Setodure' (*Psy-A1k*), *Psy-A1l* NIL or *Psy-A1o* NIL, both fragments of *Psy-E1* and *Psy-A1* were amplified only on *Psy-E1*/*A1* heterozygous (Fig. 1c). No significant difference from the expected 1:2:1 ratio was shown for any F_2 population in the same-locus model (Table 1), whereas significant differences from the expected 9:3:3:1 ratio were shown for all three F_2 populations in the different-locus model (Table 2). These results show that *Psy-E1* and *Psy-A1* lie at the same locus. *Lr19* could be amplified in individuals heterozygous for *Psy-A1* and *Psy-E1* or homozygous for *Psy-E1*, but not those homozygous for *Psy-A1*. This result is consistent with earlier studies showing that *Lr19* is closely linked to *Psy-E1*, and the linkage was unbroken in this analysis (data not shown) although other studies have shown that these genes can be separated (see Discussion).

Table 1
Segregation analysis with same-locus model in F_2 populations of *Lr19* NIL crossed with 'Setodure' or *Psy-A1* NILs.

Cross	*N*	Segregation			Chi-squared value*	*p*-value
		Psy-E1	*Psy-E1*/*Psy-A1*	*Psy-A1*		
		homozygous	heterozygous	homozygous		
Lr19 NIL × Setodure (*Psy-A1k*)	267	71	122	74	2.049	0.359
Lr19 NIL × *Psy-A1l* NIL	237	59	114	60	0.116	0.944
Lr19 NIL × *Psy-A1o* NIL	258	56	135	67	1.412	0.493

*Expected ratio of *Psy-E1* homozygous : *Psy-E1*/*Psy-A1* heterozygous : *Psy-E1* homozygous = 1:2:1 was tested by chi-squared test.

Table 2
Segregation analysis with different-locus model in F_2 population of *Lr19* NIL crossed with 'Setodure' or *Psy-A1* NILs.

Cross	N	Segregation				Chi-squared value*	*p*-value
		Both *Psy-E1* and *A1*	Only *Psy-E1*	Only *Psy-A1*	Neither *Psy-E1* nor *A1*		
Lr19 NIL × Setodure (*Psy-A1k*)	267	122	71	74	0	42.180	3.67E-09
Lr19 NIL × *Psy-A1l* NIL	237	114	59	60	0	28.242	3.23E-06
Lr19 NIL × *Psy-A1o* NIL	258	135	56	67	0	25.204	1.40E-05

*Expected ratio of both *Psy-E1* and *Psy-A1* : only *Psy-E1* : only *Psy-A1* : neither *Psy-E1* nor *Psy-A1* = 9:3:3:1 was tested by chi-squared test.

Agronomic trait measurement

Our study was conducted in Japan, where the period of wheat maturation to harvest overlaps with the rainy season with moisture stress .

To evaluate the effects of *Psy* genotype on phenotypes other than yellowness, we tested the characteristics and performance of 'Setodure' and the NILs in a 2-year field trial. No significant differences were seen among 'Setodure', *Psy-A1l* NIL and *Psy-A1o* NIL in any of the investigated agronomic traits (Table 3). Yield, grain number, volume weight and thousand-kernel weight were significantly lower in *Lr19* NIL than in the other lines (Table 3), whereas culm length and protein content of *Lr19* NIL were significantly higher. Other traits were not significantly different between *Lr19* NIL and the other lines.

Table 3
Field, spike and grain traits in 2021 and 2022.

Field traits										
	Heading date		Maturity date		Spike number		Yield		Culm length	
	(days)		(days)		(/m^2)		(kg/a)		(cm)	
2021										
Setodure	153.5 ± 0.29	a	203.0 ± 0.41	a	421.1 ± 21.20	a	55.1 ± 0.61	a	81.6 ± 0.59	a
Psy-A1/ NIL	153.3 ± 0.25	a	203.3 ± 0.25	a	454.3 ± 19.26	a	61.1 ± 2.66	a	80.0 ± 0.84	a
Psy-A1o NIL	153.3 ± 0.25	a	202.5 ± 0.29	a	471.4 ± 36.99	a	54.5 ± 2.35	a	81.0 ± 0.76	a
Lr19 NIL	154.0 ± 0.00	a	203.8 ± 0.25	a	430.0 ± 13.86	a	48.0 ± 1.00	b	85.2 ± 1.06	b
2022										
Setodure	146.8 ± 0.48	a	201.8 ± 0.25	a	389.3 ± 13.07	a	51.7 ± 0.99	a	83.7 ± 0.95	a
Psy-A1/ NIL	145.3 ± 0.63	a	200.8 ± 0.48	a	400.7 ± 13.32	a	56.2 ± 1.04	a	83.4 ± 0.78	a
Psy-A1o NIL	145.5 ± 0.29	a	200.8 ± 0.25	a	432.5 ± 20.75	a	54.5 ±2.31	a	84.8 ± 1.16	a
Lr19 NIL	146.8 ± 0.48	a	200.5 ± 0.29	a	414.7 ± 33.04	a	43.3 ± 1.26	b	91.1 ± 0.80	b
Spike traits										
	Spike length		Awn length		Spikelet number		Spikelet density*		Grain number	
	(cm)		(cm)		(/spike)				(/spike)	
2021										
Setodure	9.2 ± 0.22	a	6.9 ± 0 .44	a	22.9 ± 0.48	a	2.5 ± 0.08	a	73.6 ± 2.43	a
* Spikelet density = Spikelet number / Spike length										
Values are mean ± SE at 4 replicates (averages of 2 areas for spike number, 10 culms for culm length, and 8 spikes for spike traits). Within the same column, data marked with the same letter are not significantly different by Tukey's HSD at $P < 0.05$.										

Field traits										
Psy-A1/ NIL	9.1 ± 0.11	a	6.0 ± 0.25	a	24.8 ± 0.65	a	2.7 ± 0.08	a	72.9 ± 1.98	a
Psy-A1o NIL	9.0 ± 0.31	a	7.5 ± 0.42	a	22.0 ± 0.60	a	2.4 ± 0.07	a	73.5 ± 2.31	a
Lr19 NIL	9.3 ± 0.35	a	6.6 ± 0.39	a	24.1 ± 0.72	a	2.6 ± 0.13	a	62.8 ± 1.33	b
2022										
Setodure	9.2 ± 0.36	a	6.9 ± 0.54	a	23.4 ± 0.38	a	2.6 ± 0.11	a	71.0 ± 3.05	a
Psy-A1/ NIL	9.5 ± 0.23	a	6.4 ± 0.56	a	24.1 ± 0.48	a	2.6 ± 0.06	a	73.3 ± 1.78	a
Psy-A1o NIL	9.2 ± 0.32	a	7.3 ± 0.35	a	22.4 ± 0.32	a	2.4 ± 0.12	a	72.5 ± 1.97	a
Lr19 NIL	9.0 ± 0.22	a	6.2 ± 0.48	a	23.6 ± 0.46	a	2.6 ± 0.08	a	60.4 ± 2.27	b
Grain traits										
	Protein content		Volume weight		Diameter		Thousand-kernel weight		Hardness	
	(%)		(g/L)		(mm)		(g)		(Hardness Index)	
2021										
Setodure	11.3 ± 0.12	a	862.9 ± 2.19	a	3.3 ± 0.02	a	52.5 ± 0.84	a	93.1 ± 1.20	a
Psy-A1/ NIL	11.0 ± 0.14	a	863.5 ± 2.24	a	3.2 ± 0.02	a	51.6 ± 0.51	a	93.7 ± 0.76	a
Psy-A1o NIL	11.3 ± 0.18	a	862.3 ± 2.06	a	3.3 ± 0.02	a	52.1 ± 1.15	a	92.8 ± 0.63	a
Lr19 NIL	12.1 ± 0.22	b	852.6 ± 2.27	b	3.2 ± 0.01	a	45.7 ± 0.56	b	93.1 ± 1.03	a
2022										
Setodure	12.2 ±	a	846.2 ±	a	3.2 ±	a	51.9 ±	a	91.4 ±	a

* Spikelet density = Spikelet number / Spike length

Values are mean ± SE at 4 replicates (averages of 2 areas for spike number, 10 culms for culm length, and 8 spikes for spike traits). Within the same column, data marked with the same letter are not significantly different by Tukey's HSD at $P < 0.05$.

Field traits										
	0.21		3.41		0.02		0.68		1.27	
Psy-A1l NIL	11.9 ± 0.26	a	851.5 ± 2.85	a	3.3 ± 0.00	a	51.7 ± 0.22	a	90.4 ± 0.44	a
Psy-A1o NIL	11.8 ± 0.24	a	849.5 ± 2.46	a	3.2 ± 0.02	a	51.2 ± 0.78	a	90.4 ± 0.95	a
Lr19 NIL	13.2 ± 0.25	b	830.7 ± 2.84	b	3.2 ± 0.02	a	45.3 ± 0.91	b	90.9 ± 0.55	a
* Spikelet density = Spikelet number / Spike length										
Values are mean ± SE at 4 replicates (averages of 2 areas for spike number, 10 culms for culm length, and 8 spikes for spike traits). Within the same column, data marked with the same letter are not significantly different by Tukey's HSD at $P < 0.05$.										

Discussion

The chromosome segment containing *Lr19*, derived from the wild relative *Th. ponticum*, confers resistance to leaf rust in wheat (Gennaro et al. 2009) and increases the yellowness in seeds, but the degree of its effect on yellowness in durum wheat was unknown. In this study, yellowness as measured by YPC and b* values in multiple years and in different sample types decreased in the order of *Lr19* NIL > *Psy-A1o* NIL > *Psy-A1l* NIL > 'Setodure' (*Psy-A1k*), (Figs. 2, 3). Singh et al. (2009) reported that b* value decreased in the order of *Psy-A1l* > *Psy-A1o* > *Psy-A1a* in two panels as modern cultivars and landraces in durum wheat, but no significant difference between *Psy-A1l* and *Psy-A1o* under some conditions. *Lr19*-possessing lines were generated in several studies, and their seeds and flour showed strong yellowness (Prins et al. 1997; Rai et al. 2019; Zhang et al. 2005). This is the first report that the yellowness conferred by *Lr19* is higher than that conferred by any of the *Psy-A1* alleles in durum wheat.

Lr19 is linked with *Y* on Chr. 7E from *Th. ponticum*, which confers yellow pigmentation in endosperm (Ayala-Navarrete et al. 2013; Sarma and Knott 1966; Zhang and Dubcovsky 2008). Here, *Lr19* NIL had high YPC and carries *Psy-E1* (Fig. 1–3), consistent with previous research. Interestingly, *Lr19* NIL did not show amplification of any *Psy-A1*–specific amplicons and only F_1 crossing *Lr19* NIL with 'Setodure' (*Psy-A1k*) or *Psy-A1* NILs show both *Psy-A1*–specific and *Psy-E1*–specific amplicons (Fig. 1). In addition, both fragments of *Psy-E1* and *Psy-A1* were detected only on *Psy-E1*/*A1* heterozygous in F_2 populations. Taking these fact into consideration, we clarified by using segregation analysis that *Psy-E1* is not tandem arrangement with *Psy-A1*, but is located at the *Psy-A1* locus (Table 1). These results provide further evidence that *Psy-E1* is a candidate for the *Y* gene linked with *Lr19*.

We observed strong linkage between *Lr19* and *Psy-E1*, consistent with previous research (Knott 1980; Marais and Marais 1990). This linkage block was not broken in this study (Table 1), however, Prins et al. (1997) revealed that white-endosperm lines with *Lr19* lacked the *Y* locus from a translocated segment. Xu et al. (2023) also succeeded in breaking this linkage with homoeologous recombination and

established a white-endosperm line lacking *Psy-E1* but carrying *Lr19*. But because *Psy-E1* lies at the same locus as *Psy-A1*, wheat breeders would have to choose between the *Psy-E1* and *Psy-A1* alleles for controlling yellowness.

Here, *Lr19* NIL had both higher culm length and lower yield than the *Psy-A1*–containing lines, with lower grain number per spike, volume weight and thousand-kernel weight than the recurrent durum wheat parent 'Setodure' and *Psy-A1* NILs in the 'Setodure' genetic background (Table 3). In bread wheat, yield was increased in *Lr19*-containing NILs in various backgrounds in India (Rai et al. 2019) and Mexico (Reynolds et al. 2001). The yield and number of grains per m^2 were increased in *Lr19*-containing NILs under appropriate irrigated conditions compered to under drought conditions (Monneveux et al. 2003) and yield increase with biomass and number of spikes per m2 elevate was observed under non-moisture conditions with appropriate irrigations compared to under moisture conditions (Singh et al. 1998). They concluded that the translocation of Chr. 7E may be useful for enhancing yield under favorable conditions (Monneveux et al. 2003; Singh et al. 1998). Monneveux et al. (2003) pointed out that agronomic phenotypes of the effect of the Chr. 7E translocation depend on the recipient genotype. Thus, the agronomic potential and effectiveness of the 7E segment including *Lr19* and *Psy-E1* will need to be assessed under appropriate cultivation conditions and in various durum wheat backgrounds.

Functional characterization of PSY in carotenogenesis has revealed various regulatory stages acting at the transcriptional, post-transcriptional, post-translational and epigenetic levels (Zhou et al. 2022). The effects of engineering the carotenoid biosynthetic pathway through manipulation of *Psy1* have been researched in many plants, because PSY is a highly conserved enzyme that catalyzes the first step in the carotenoid biosynthesis pathway and is a major rate-limiting enzyme (Cong et al. 2009; Lindgren et al. 2003; Zhou et al. 2022).

Alternative splicing occurs in *Psy-A1* owing to duplication in an exon, producing aberrant transcripts and decreasing the yellow color of endosperm in bread wheat (Howitt et al. 2009). Cultivars of durum and bread wheat with strong yellow endosperm had higher *Psy1* gene expression (Qin et al. 2016). Tissue-specific expression and overexpression of bacterial PSY genes dramatically increased provitamin A in bread wheat (Wang et al. 2014). However, functional characterization of *Psy-A1* alleles and *Psy-E1* in wheat is still incomplete, and the effects of these alleles need to be better understood to lead to effective durum wheat breeding in the future.

In this study, *Lr19*-linked *Psy-E1* conferred higher yellowness in endosperm, a trait desirable for pasta production. However, it was accompanied by yield loss under the field conditions in this study, which were typical for wheat-growing areas of Japan. Among the future challenges for durum wheat breeding will be to break the linkage between *Psy-E1* and yield loss through backcrossing to enable application of *Psy-E1* in breeding programs, and to explore the combination of *Psy-A1* and *Psy-B1* in durum wheat to produce yellowness as strong as that conferred by *Lr19*.

Declarations

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Author contributions

Keita Kato: Conceptualization, data curation, investigation, resources, validation, visualization, writing original draft. Yusuke Ban: Data curation, investigation, validation. Mikiko Yanaka, Wakako Funatsuki and Kanenori Takata: Resources. Hideki Okusu, Tomoki Tanaka, Hiroyuki Kawakami and Masahiro Yamaguchi: Data curation, validation. Miwako Ito: Conceptualization, data curation, investigation, validation. All authors read and approved the final manuscript.

Data availability

The data supporting the findings of this study are available from the corresponding author, Miwako Ito, upon request.

The sponsor had no control over the interpretation, writing, or publication of this work.

Conflict of interest

This study was funded by NIPPN Corporation. Hideki Okusu, Tomoki Tanaka, Hiroyuki Kawakami and Masahiro Yamaguchi are employees of NIPPN corporation.

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References

1. AACC Approved Methods of Analysis, 11th Ed. Method 14-50.01. Determination of Pigments. Approved November 3, 1999a. Cereals & Grains Association, St. Paul, MN, U.S.A. http://dx.doi.org/10.1094/AACCIntMethod-14-50.01
2. AACC Approved Methods of Analysis, 11th Ed. Method 26-41.01. Experimental Milling-Bühler Method for Durum Wheat. Approved November 3, 1999b. Cereals & Grains Association, St. Paul, MN, U.S.A. http://dx.doi.org/10.1094/AACCIntMethod-26-41.01
3. AACC Approved Methods of Analysis, 11th Ed. Method 66-42.01. Micro Scale Pasta Processing. Approved November 3, 1999c. Cereals & Grains Association, St. Paul, MN, U.S.A. http://dx.doi.org/10.1094/AACCIntMethod-66-42.01
4. AACC Approved Methods of Analysis, 11th Ed. Method 66-50.01. Pasta and Noodle Cooking Quality-Firmness. Approved November 3, 1999d. Cereals & Grains Association, St. Paul, MN, U.S.A. http://dx.doi.org/10.1094/AACCIntMethod-66-50.01

5. Abdel-Aal E-SM, Young JC, Rabalski I, et al (2007) Identification and quantification of seed carotenoids in selected wheat species. J Agric Food Chem 55:787–794. https://doi.org/10.1021/jf062764p
6. Albrecht M, Klein A, Hugueney P, et al (1995) Molecular cloning and functional expression in *E. coli* of a novel plant enzyme mediating ξ-carotene desaturation. FEBS Lett 372:199–202. https://doi.org/10.1016/0014-5793(95)00978-I
7. Ayala-Navarrete LI, Mechanicos AA, Gibson JM, et al (2013) The Pontin series of recombinant alien translocations in bread wheat: single translocations integrating combinations of *Bdv2*, *Lr19* and *Sr25* disease-resistance genes from *Thinopyrum intermedium* and *Th. ponticum*. Theor Appl Genet 126:2467–2475. https://doi.org/10.1007/s00122-013-2147-0
8. Bartley GE, Scolnik PA, Beyer P (1999) Two *Arabidopsis thaliana* carotene desaturases, phytoene desaturase and ζ-carotene desaturase, expressed in *Escherichia coli* , catalyze a poly- *cis* pathway to yield pro-lycopene. Eur J Biochem 259:396–403. https://doi.org/10.1046/j.1432-1327.1999.00051.x
9. Campos KM, Royo C, Schulthess A, et al (2016) Association of phytoene synthase *Psy1-A1* and *Psy1-B1* allelic variants with semolina yellowness in durum wheat (*Triticum turgidum* L. var. *durum*). Euphytica 207:109–117. https://doi.org/10.1007/s10681-015-1541-x
10. Clarke FR, Clarke JM, McCaig TN, et al (2006) Inheritance of yellow pigment concentration in seven durum wheat crosses. Can J Plant Sci 86:133–141. https://doi.org/10.4141/P05-083
11. Cong L, Wang C, Chen L, et al (2009) Expression of *phytoene synthase1* and Carotene Desaturase *crtI* Genes Result in an Increase in the Total Carotenoids Content in Transgenic Elite Wheat (*Triticum aestivum* L.). J Agric Food Chem 57:8652–8660. https://doi.org/10.1021/jf9012218
12. Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: Version II. Plant Mol Biol Rep 1:19–21. https://doi.org/10.1007/BF02712670
13. Dibari B, Murat F, Chosson A, et al (2012) Deciphering the genomic structure, function and evolution of carotenogenesis related phytoene synthases in grasses. BMC Genom 13:221. https://doi.org/10.1186/1471-2164-13-221
14. Digesù AM, Platani C, Cattivelli L, et al (2009) Genetic variability in yellow pigment components in cultivated and wild tetraploid wheats. J Cereal Sc 50:210–218. https://doi.org/10.1016/j.jcs.2009.05.002
15. Ficco DBM, Mastrangelo AM, Trono D, et al (2014) The colours of durum wheat: a review. Crop Pasture Sci 65:1. https://doi.org/10.1071/CP13293
16. Fraser PD, Bramly PM (2004) The biosynthesis and nutritional uses of carotenoids. Prog Lipid Res 43:228–265. https://doi.org/10.1016/j.plipres.2003.10.002
17. Gennaro A, Koebner RMD, Ceoloni C (2009) A candidate for *Lr19*, an exotic gene conditioning leaf rust resistance in wheat. Funct. Integr. Genomics 9:325–334. https://doi.org/10.1007/s10142-009-0115-1

18. He X, Wang J, Ammar K, et al (2009) Allelic variants at the and loci in durum wheat and their associations with grain yellowness. Crop Sci 49:2058. https://doi.org/10.2135/cropsci2008.11.0651
19. Hentschel V, Kranl K, Hollmann J, et al (2002) Spectrophotometric determination of yellow pigment content and evaluation of carotenoids by high-performance liquid chromatography in durum wheat grain. J Agric Food Chem 50:6663–6668. https://doi.org/10.1021/jf025701p
20. Howitt CA, Cavanagh CR, Bowerman AF, et al (2009) Alternative splicing, activation of cryptic exons and amino acid substitutions in carotenoid biosynthetic genes are associated with lutein accumulation in wheat endosperm. Funct Integr Genomics 9:363–376. https://doi.org/10.1007/s10142-009-0121-3
21. Kato K, Maruyama-Funatsuki W, Yanaka M, et al (2017) Improving preharvest sprouting resistance in durum wheat with bread wheat genes. Breeding Sci 67:466–471. https://doi.org/10.1270/jsbbs.17042
22. Knott DR (1980) Mutation of a gene for yellow pigment linked to *Lr19* in wheat. Can J Genet Cytol 22:651–654. https://doi.org/10.1139/g80-071
23. Lachman J, Martinek P, Kotíková Z, et al (2017) Genetics and chemistry of pigments in wheat grain – A review. J Cereal Sc 74:145–154. https://doi.org/10.1016/j.jcs.2017.02.007
24. Li F, Vallabhaneni R, Wurtzel ET (2008) *PSY3*, a new member of the phytoene synthase gene family conserved in the Poaceae and regulator of abiotic stress-induced root carotenogenesis. Plant Phys 146:1333–1345. https://doi.org/10.1104/pp.107.111120
25. Lindgren LO, Stålberg KG, Höglund A-S (2003) Seed-specific overexpression of an endogenous arabidopsis phytoene synthase gene results in delayed germination and increased levels of carotenoids, chlorophyll, and abscisic acid. Plant Phys 132:779–785. https://doi.org/10.1104/pp.102.017053
26. Marais GF, Marais AS (1990) The assignment of a *Thinopyrum distichum* (Thunb.) Löve-derived translocation to the long arm of wheat chromosome *7D* using endopeptidase polymorphisms. Theor Appl Genet 79:182–186. https://doi.org/10.1007/BF00225949
27. Matthews PD (2003) Maize phytoene desaturase and -carotene desaturase catalyze a poly-Z desaturation pathway: implications for genetic engineering of carotenoid content among cereal crops. J Exp Bot 54:2215–2230. https://doi.org/10.1093/jxb/erg235
28. McCallum BD, Hiebert CW, Cloutier S, et al (2016) A review of wheat leaf rust research and the development of resistant cultivars in Canada. Can J Plant Pathol 38:1–18. https://doi.org/10.1080/07060661.2016.1145598
29. Monneveux P, Reynolds MP, Aguilar JG, et al (2003) Effects of the 7DL.7Ag translocation from *Lophopyrum elongatum* on wheat yield and related morphophysiological traits under different environments. Plant Breed 122:379–384. https://doi.org/10.1046/j.1439-0523.2003.00856.x
30. Pozniak CJ, Knox RE, Clarke FR, Clarke JM (2007) Identification of QTL and association of a phytoene synthase gene with endosperm colour in durum wheat. Theor Appl Genet 114:525–537. https://doi.org/10.1007/s00122-006-0453-5

31. Prasad P, Savadi S, Bhardwaj SC, et al (2020) The progress of leaf rust research in wheat. Fungal Biol 124:537–550. https://doi.org/10.1016/j.funbio.2020.02.013
32. Prins R, Marais GF, Pretorius ZA, et al (1997) A study of modified forms of the *Lr19* translocation of common wheat: Theor Appl Genet 95:424–430. https://doi.org/10.1007/s001220050579
33. Qin X, Fischer K, Dubcovsky J, Tian L (2016) Endosperm Carotenoid Concentrations in Wheat are Better Correlated with *PSY1* Transcript Levels than Enzyme Activities. Crop Sci 56:3173–3184. https://doi.org/10.2135/cropsci2016.06.0520
34. Rai A, Mahendru-Singh A, Ahlawat AK, et al (2019) Quality evaluation of near isogenic lines of the wheat variety carrying *Sr26*, *Lr19* and *Yr10* genes. J Cereal Sci 88:110–117. https://doi.org/10.1016/j.jcs.2019.05.009
35. Ramachandran A, Pozniak CJ, Clarke JM, Singh AK (2010) Carotenoid accumulation during grain development in durum wheat. J Cereal Sci 52:30–38. https://doi.org/10.1016/j.jcs.2010.02.014
36. R Core Team (2023) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
37. Reynolds MP, Calderini DF, Condon AG, Rajaram S (2001) Physiological Basis of Yield Gains in Wheat Associated with the *Lr19* Translocation from *Agropyron elongatum*. Euphytica 119:137–141. https://doi.org/10.1007/978-94-017-3674-9_44
38. Sarma D, Knott DR (1966) The transfer of leaf-rust resistance from *Agropyron* to *Triticum* by irradiation. Can J Genet Cytol 8:137–143. https://doi.org/10.1139/g66-018
39. Saito M, Vrinten P, Ishikawa G, et al (2009) A novel codominant marker for selection of the null *Wx-B1* allele in wheat breeding programs. Mol Breed 23:209–217. https://doi.org/10.1007/s11032-008-9226-y
40. Singh A, Reimer S, Pozniak CJ, et al (2009) Allelic variation at Psy1-A1 and association with yellow pigment in durum wheat grain. Theor Appl Genet 118:1539–1548. https://doi.org/10.1007/s00122-009-1001-x
41. Singh RP, Huerta-Espino J, Rajaram S, Crossa J (1998) Agronomic Effects from Chromosome Translocations 7DL.7Ag and 1BL.1RS in Spring Wheat. Crop Sci 38:27–33. https://doi.org/10.2135/cropsci1998.0011183X003800010005x
42. Wang C, Zeng J, Li Y, et al (2014) Enrichment of provitamin A content in wheat (*Triticum aestivum* L.) by introduction of the bacterial carotenoid biosynthetic genes CrtB and CrtI. J Exp Bot 65:2545–2556. https://doi.org/10.1093/jxb/eru138
43. Xu S, Lyu Z, Zhang N, et al (2023) Genetic mapping of the wheat leaf rust resistance gene *Lr19* and development of translocation lines to break the linkage with *PSY-E1*. PREPRINT (Version 1) available at Research Square. https://doi.org/10.21203/rs.3.rs-2501575/v1
44. Zhang W, Dubcovsky J (2008) Association between allelic variation at the Phytoene synthase 1 gene and yellow pigment content in the wheat grain. Theor Appl Genet 116:635–645. https://doi.org/10.1007/s00122-007-0697-8

45. Zhang W, Lukaszewski AJ, Kolmer J, et al (2005) Molecular characterization of durum and common wheat recombinant lines carrying leaf rust resistance (*Lr19*) and yellow pigment (*Y*) genes from *Lophopyrum ponticum*. Theor Appl Genet 111:573–582. https://doi.org/10.1007/s00122-005-2048-y
46. Zhou X, Rao S, Wrightstone E, et al (2022) Phytoene Synthase: The Key Rate-Limiting Enzyme of Carotenoid Biosynthesis in Plants. Front Plant Sci 13:884720. https://doi.org/10.3389/fpls.2022.884720

Figures

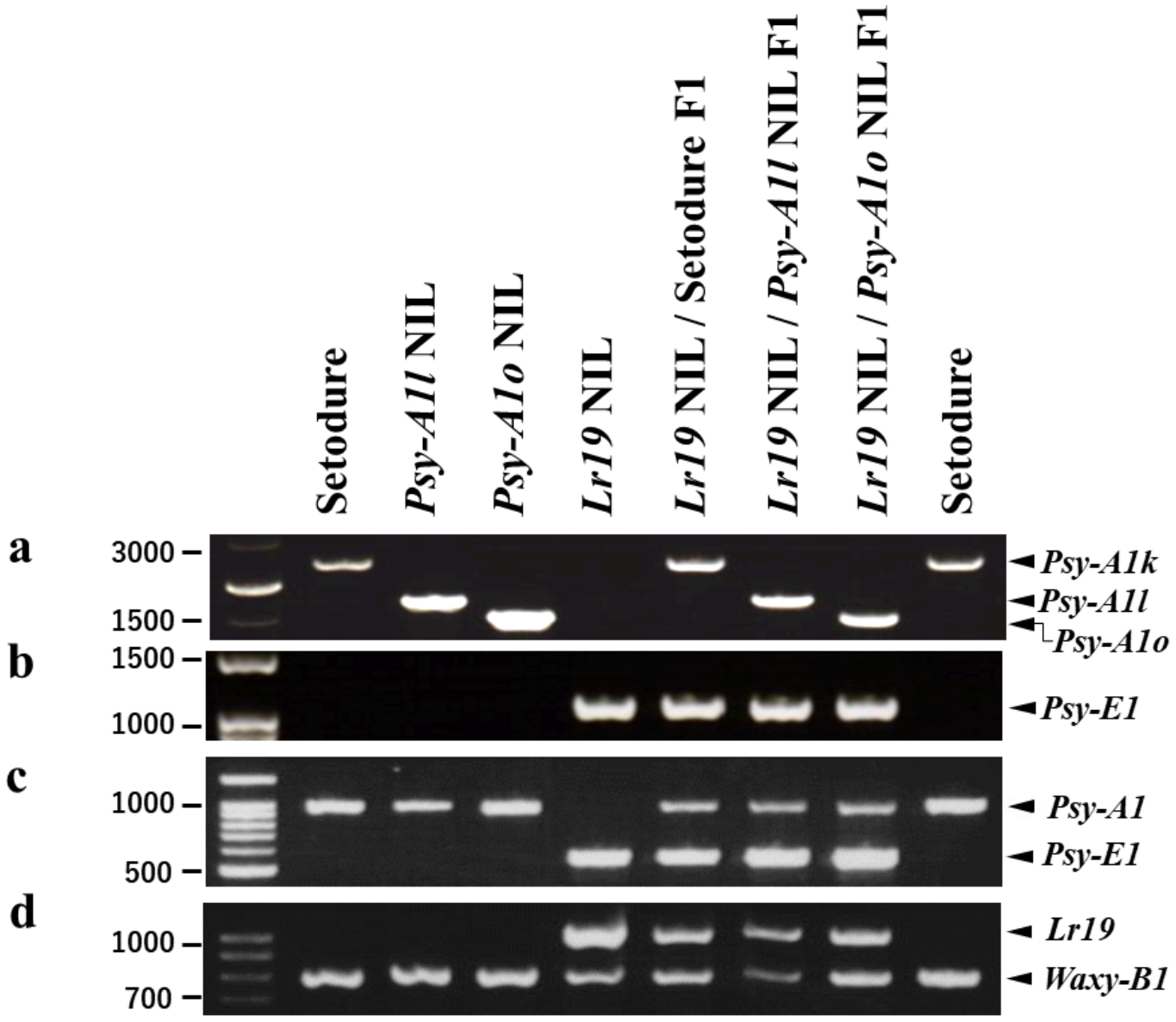


Figure 1

Genotyping of materials containing *Psy-A1*, *Psy-E1* and *Lr19*. Templates were 'Setodure', 3 NILs, and F_1 hybrids of *LR19* NIL × 'Setodure' and of *LR19* NIL *× Psy-A1* NILs. **a** Codominant marker detecting *Psy-A1*

alleles. **b** Dominant *Psy-E1*–specific marker. **c** Codominant marker detecting *Psy-A1* and *Psy-E1*. **d** Dominant *Lr19*–specific marker with *Waxy-B1* as internal control.

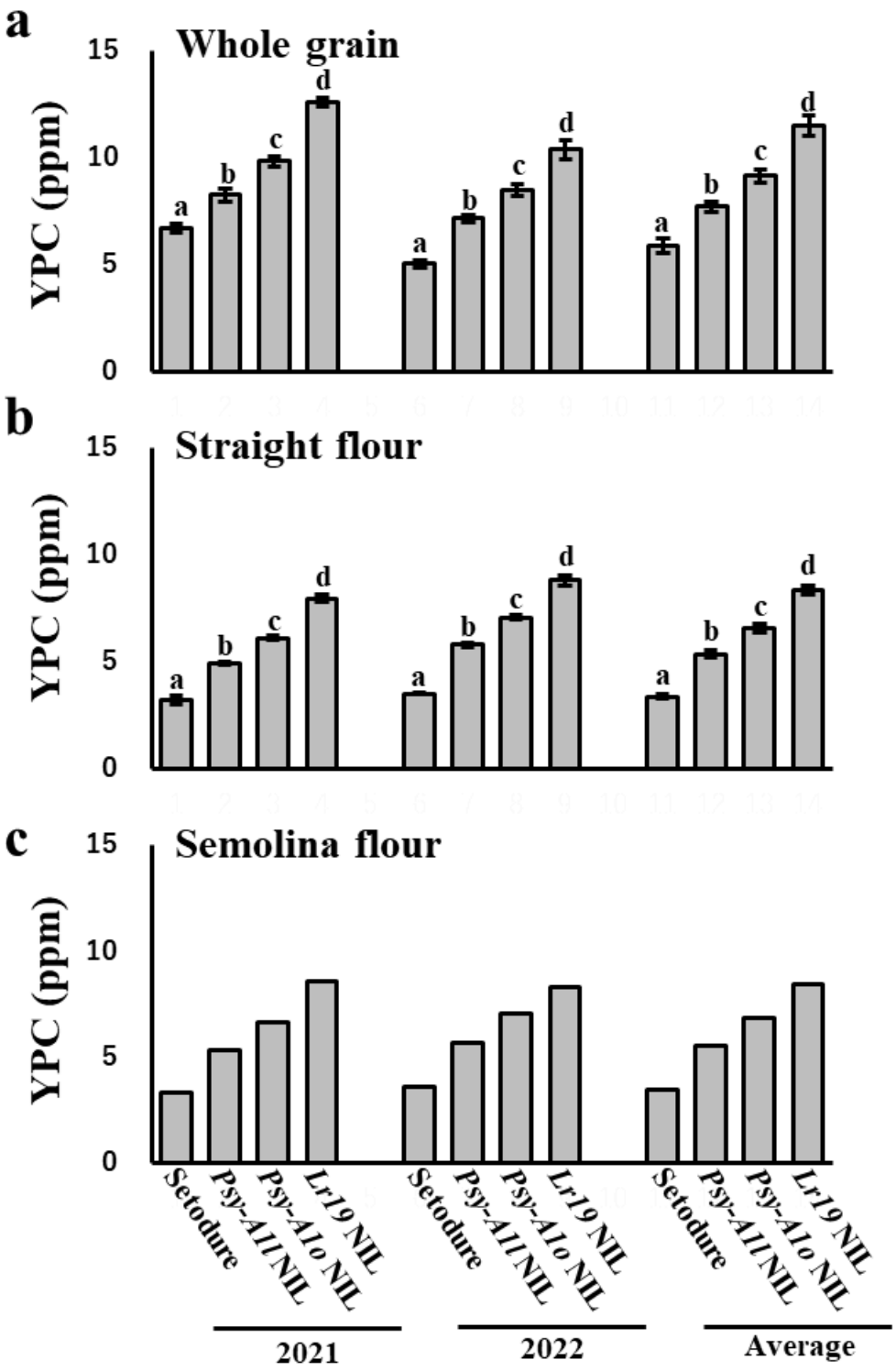


Figure 2

Yellow pigment content (YPC) of 'Setodure' and 3 NILs in 2020, 2021 and 2-year average. Sample type was (**a**) whole grain, (**b**) straight flour and (**c**) semolina flour. Values were means ± SE (*n*= 4 in 2021 and

2022, *n* = 8 in 2-year average). Same letters indicate no significantly differences by Tukey's HSD at *P* < 0.05.

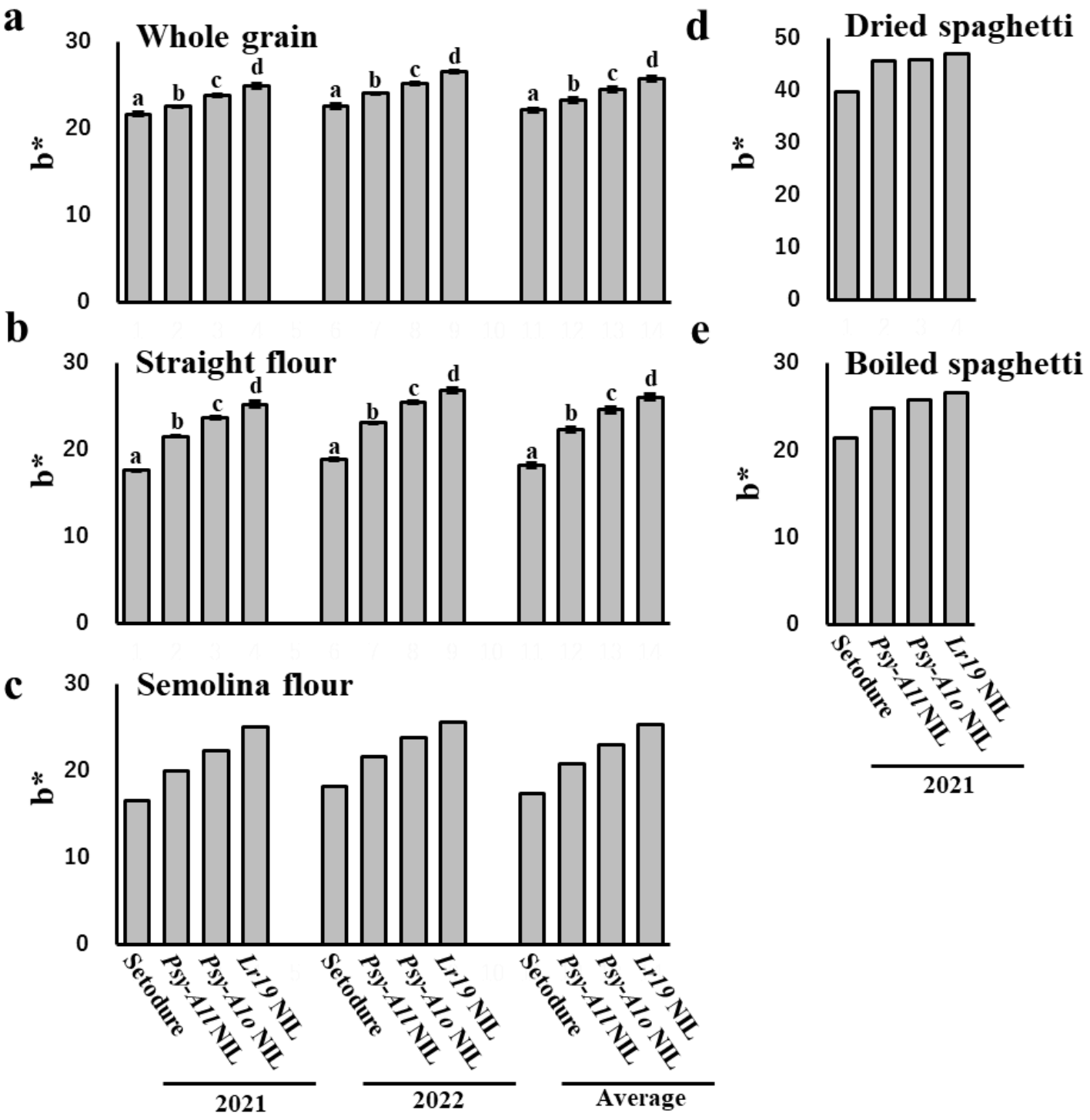


Figure 3

b*value of 'Setodure' and 3 NILs in 2020, 2021 and 2-year average. Sample type was (**a**) whole grain, (**b**) straight flour, (**c**) semolina flour, (**d**) dried spaghetti and (**e**) boiled spaghetti. Values are means ± SE (**a**–**c**: *n* = 4 in 2021 and 2022, *n* = 8 in 2-year average) and mean (**d**, **e**: 4 replicates were mixed together in). Same letters in **a**–**c** indicate no significantly difference by Tukey's HSD at *P* < 0.05.

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