This is a postprint of the article by Ávila CM, Palomino MC, Hornero-Méndez D and Atienza SG. Identification of candidate genes for lutein esterification in wheat (*Triticum aestivum* L.) using physical mapping and genomics tools.

*Crop & Pasture Science Online early.*

The definitive version of this article is available on the journal's website.

[https://doi.org/10.1071/CP18531](https://doi.org/10.1071/CP18531)
Identification of candidate genes for lutein esterification in wheat (*Triticum aestivum* L.) using physical mapping and genomics tools

Ávila CM¹, Palomino MC³, Hornero-Méndez D², Atienza SG³

¹ Área Mejora y Biotecnología, IFAPA-Centro Alameda del Obispo, Apdo. 3092, 14080, Córdoba, Spain

² Departament of Food Phytochemistry, Instituto de la Grasa (CSIC). Campus Universidad Pablo de Olavide, Edificio 46. Ctra. de Utrera, Km 1, E-41013 Sevilla, Spain.

³ Instituto de Agricultura Sostenible-CSIC, Alameda del Obispo s/n, 14004, Córdoba, Spain

*Corresponding author

E-mail: SG Atienza: sgatienza@ias.csic.es

Phone: +34 957499260
Abstract

A high carotenoid content is important for the production of pasta from durum wheat and yellow alkaline noodle from common wheat. Carotenoid esters are more stable than free carotenoid during storage and processing and thus they allow a higher retention through the food chain.

Chromosome 7D carries gene(s) for lutein esterification. The aim of this study was the physical mapping of the gene(s) for lutein esterification in chromosome 7D and the identification of candidate genes for this trait. To do this we developed crosses between a set of deletion lines for chromosome 7D in Chinese Spring (CS) background and the CS-Hordeum chilense substitution line CS(7D)7Hch. The F$_2$ progeny derived from the deletion line 7DS4 produced lower amount of lutein esters which indicates that the main gene for lutein esterification is in the region of chromosome 7D lacking in 7DS4. Other gene(s) are contributing to lutein esterification since small amounts of lutein esters are produced in 7DS4.

DArTSeq genotyping revealed that 7DS4 lacks a 127.7 Mb region of 7DS. A set of 10 candidate genes for lutein esterification was identified using the wheat reference genome sequence along with the Wheat Expression Browser. This region contains the Lute locus previously identified in a different genetic background. Four genes with acyltransferase or GDSL_esterase/lipase activity were identified in the vicinity of Lute. Our results indicate that the gene TraesCS7D01g094000 is a likely candidate for Lute but the gene TraesCS7D01g093200 cannot be ruled out. The candidate genes reported in this work are worthy for further investigation.
Introduction

Carotenoids pigments, mostly lutein, are the main responsible for the yellow colour (YC) of endosperm of wheat and related cereals. YC is a significant criterion for durum wheat breeding since a bright yellow colour is required for the production of pasta (Ficco et al. 2014). Besides, YC is also important for the fabrication of yellow alkaline noodles (Mares and Campbell 2001) and thus a higher lutein content in common wheat would be beneficial (Wijaya et al. 2016).

Lutein esterification may be useful to improve lutein retention through the food chain since lutein esters are more stable than free lutein (Subagio et al. 1999; Ahmad et al. 2013; Mellado-Ortega et al. 2015). Common wheat and durum wheat are able to produce lutein esters in the endosperm (Atienza et al. 2007; Ahmad et al. 2015; Paznocht et al. 2018) but only common wheat is able to produce lutein diesters in intact grains (Atienza et al. 2007; Mellado-Ortega et al. 2015).

Genetic studies on carotenoid esterification have shown the role of acyltransferases and esterases in several species. Lippold et al. (2012) reported that the genes *Phylit ester synthase1* (*PES1*) and *PES2* are involved in phytol fatty acid esters synthesis in chloroplast of *Arabidopsis*. These genes belong to the esterase/lipase/thioesterase family of acyltransferases (Lippold et al. 2012). Similarly, the gene *PYP1* (*Pale Yellow Petal*) is involved in carotenoid esterification in tomato flowers (Ariizumi et al. 2014). This gene harbors two different domains, an acyltransferase domain and a hydrolase domain (Ariizumi et al. 2014) while the gene *AtSAT1* (*At3g51970*) encodes a sterol-O-acyltransferase which is involved in the synthesis of phytoesterol-esters (Chen et al. 2007). Likewise, GDSL esterases have been reported to have potential for synthesis of ester compounds (Akoh et al. 2004).

Little is known of the genetic bases of lutein esterification in wheat and related cereals. Kaneko et al. (1995) proposed that lutein esterification may involve an acylhydrolase. A QTL for lutein esterification was located on chromosome 2B (Howitt et al. 2009). However, the main genes for
lutein esterification were located in chromosomes 7D and 7Hch using a set of common wheat-
Hordeum chilense chromosome substitution lines (Mattera et al. 2015) and in chromosome 7DS
(Lute locus) using doubled haploid lines derived from the cross Haruhikari/Sunco/Indis.82
(Ahmad et al. 2015). It seems that H. chilense has more than one gene involved in lutein
esterification (Mattera et al. 2015) and the same may happen with common wheat. Indeed Lute
was mapped by classifying the double haploid lines as zero/low or high lutein ester content
(Ahmad et al. 2015). However, the existence of variation for lutein esterification among the lines
within each class (Ahmad et al. 2015) may indicate the existence of additional genes involved in
lutein esterification in common wheat.

Candidate genes including an acyltransferase-like protein (Mattera et al. 2015) and a GSDL-like
lipase (Ahmad et al. 2015) have been considered worthy of further investigation for lutein
esterification in wheat and related cereals. The recent release of the wheat reference genome
sequence (Alaux et al. 2018) is a powerful tool for the development of genomics studies in wheat.
It allows the identification of candidate genes underlying traits of interest considering their
putative function and their physical location. Similarly genetic stocks of common wheat are good
tools for physical mapping of wheat chromosomes (Endo and Gill 1996). The utilization of
deletion lines of common wheat ‘Chinese Spring’ (CS) for chromosome 7D would allow the
physical mapping of the gene(s) involved in lutein esterification located in this chromosome.
However, the low carotenoid content of CS constitutes a handicap for the reliable quantification of
lutein esters. Fortunately the simultaneous presence of chromosomes 7Hch and 7D increases
both the total carotenoid content and the proportion of lutein esters (Mattera et al. 2015; Mattera
et al. 2017).

Thus, the aim of this work was the physical mapping of the gene(s) for lutein esterification in
chromosome 7D and the identification of candidate genes for this trait. The present study
compared the carotenoid profile of $F_2$ progenies derived from crosses between a set of deletion
lines for chromosome 7D in CS background and the CS-\textit{H. chilense} substitution line CS(7D)7Hch. Differences in carotenoid esters along with DArTSeq genotyping allowed the identification of a region in chromosome 7DS harbouring genes for lutein esterification. Further investigation of this region in relation with the wheat reference genome and previous genetic studies allowed the identification of candidate genes for lutein esterification in wheat and related cereals.

**Material and methods**

**Plant material and experimental design**

A set of 6 deletion stocks of common wheat ‘Chinese Spring’ (CS) corresponding to chromosome 7D (Endo and Gill 1996) were used as maternal parents for crossing with the CS-\textit{H. chilense} chromosome substitution line CS(7D)7Hch (Table 1) (T.E. Miller and S.M. Reader, unpublished results; [www.jic.ac.uk/germplasm/Wheat-Precise-Genetic_stocks-Aliens.pdf](http://www.jic.ac.uk/germplasm/Wheat-Precise-Genetic_stocks-Aliens.pdf)).

Segregating progenies (F₂) for carotenoid analyses were developed as shown in Supplementary Fig. 1. Initial crosses between each deletion stock (used as mother line) and CS(7D)7Hch (pollen donor) were developed at IAS-CSIC during the 2014-2015 season. F₁ plants were checked with molecular markers for chromosomes 7D and 7Hch (see below) and F₁ spikes were bagged to develop F₂ progenies by self-pollination.

For each cross, three F₁ plants were used to develop F₂ progenies. Eighteen F₂ progenies (6 crosses × 3 plants) were analyzed. From each F₂, ninety-six seeds were selected and analyzed using the half-grain technique. The half containing the embryo was germinated on moistened filter paper in 96 well tube racks at 4 °C in the dark during four days. After this they were transferred to a growth chamber at 24/8 °C (day/night) with 12/12 hours (day/night). The remaining half grain was individually stored at -80 °C for pigment analyses (see below).
Molecular markers analyses

Total genomic DNA was isolated from young frozen leaf tissue using the CTAB method (Murray and Thompson 1980) using TissueLyser II mill (Qiagen) for sample disruption with two stainless-steel balls (5 mm diameter) in 1.2 mL collection tubes at 30 Hz for 1 min. A set of chromosome-specific single sequence repeats (SSR) markers for D genome (Xgwm295, Xcfd46 and Xbarc53) were used to check the deletion stocks and the F₁ hybrids. Primers and amplification conditions are described in GrainGenes, (https://wheat.pw.usda.gov/cgi-bin/GG3/browse.cgi?class=marker). Barley markers BAWU550 and BAWU763 (Hagras et al. 2005) were used to confirm the presence of chromosome 7Hch.

F₂ individuals were genotyped with specific markers amplifying either chromosome 7D or 7Hch as described above. For each progeny, individuals were classified into three different classes (homozygous for 7D [7D7D], homozygous for 7Hch [7Hch7Hch] or heterozygous [7D7Hch]. Within each progeny, the half-grains were pooled according to their genotype for pigment analyses but individuals homozygous for 7Hch were not analyzed since we were interested in the effect of chromosome 7D. Additionally, the deletion stocks were further genotyped using DArTSeq markers (Diversity Arrays Technology Pty Ltd, Canberra, Australia). DArTSeq sequences were used as a BLASTn query against the High Confidence (HC) and Low Confidence (LC) gene models (Alaux et al. 2018) of wheat (https://wheat-urgi.versailles.inra.fr/Seq-Repository/Annotations) using BLAST+ (Camacho et al. 2009).

Extraction of carotenoids

Carotenoid pigments were extracted from half-grains samples using the method described by Mellado-Ortega and Hornero-Méndez (2016), with slight modifications. Grain sample (from 0.2 to 0.7 g, depending on the genotype) were placed in a grinding-jar together with
with 6 mL of HPLC grade acetone (containing 0.1% BHT), and subsequently milled in an oscillating ball mill Retsch Model MM400 (Retsch, Haan, Germany) with two stainless-steel balls (10 mm Ø) at 25 Hz for 1 min. The resulting slurry was placed in a centrifuge tube (15 mL) and centrifuged at 4,500×g for 5 min at 4 ºC. The acetone phase was transferred to another plastic centrifuge tube and the solvent was evaporated under nitrogen stream. The concentrated residue containing the pigments was dissolved in 0.5 mL of HPLC grade acetone and stored at -30 ºC until chromatographic analysis (HPLC). To prevent photo-degradation of carotenoids, the whole process was carried out under dimmed light. Prior to chromatographic analysis, all the samples were centrifuged at 13,000×g.

**HPLC analyses of carotenoids**

The procedures for the identification of carotenoid pigments and their esters in cereal grains have already been described in previous works (Atienza et al. 2007; Mellado-Ortega and Hornero-Mendez 2012).

Quantitative HPLC analysis of carotenoids was carried out according to the method of Minguez-Mosquera and Hornero-Méndez (1993) with some modifications (Atienza et al. 2007). The HPLC system consisted of a Waters e2695 Alliance chromatograph fitted with a Waters 2998 photodiode array detector, and controlled with Empower2 software (Waters Cromatografía, S.A., Barcelona, Spain). A reversed-phase column (Mediterranea SEA18, 3 μm, 20×0.46 cm; Teknokroma, Barcelona, Spain) was used. Pigment separation was achieved by a binary-gradient elution using an initial composition of 75% acetone and 25% deionized water, which was increased linearly to 95% acetone in 10 min, then raised to 100% in 2 min, and maintained constant for 10 min. Initial conditions were reached in 5 min. An injection volume of 20 μL and a flow rate of 1 mL/min were used. Detection was performed at 450 nm, and the online spectra were acquired in the 350-700 nm wavelength range. Quantification was carried out using...
calibration curves prepared with lutein, α- and β-carotene and zeaxanthin standards at concentration range of 0.5-45 μg/ml. Since the esterification of xanthophylls with fatty acids does not modify the chromophore properties, lutein esters contents were estimated by using the calibration curve for free lutein, thus the concentration of lutein esters was expressed as free lutein equivalents. The calibration curve of free lutein was also used to determine the concentration of the cis-isomers of lutein. Data were expressed as μg/g fresh weight. The proportion of lutein esters relative to the total carotenoid pool was determined and used for comparison among the different genetic stocks.

Results and discussion
Crosses between each of the deletion stocks of CS (7DS4, 7DL2, 7DL4, 7DL5, 7DL6 and 7DL8) and the CS-H. chilense chromosome substitution line CS(7D)7Hch were developed. Thus, all these progenies share the CS background but they differ for the deletion of chromosome 7D. F₁ hybrids were checked for chromosomes 7D and 7Hch using chromosome-specific markers before the development of F₂ progenies by selfing.
A total of 1,728 F₂ individuals (288 from each cross) were genotyped with 7D- and 7Hch-specific markers. The majority of the F₂ populations did not fit to mendelian inheritance (Table 2). This was expected since F₁ hybrids carried a deleted 7D and an alien 7Hch chromosome. Only the progeny derived from the cross 7DL6 × DS7Hch(7D) fitted to mendelian inheritance (Table 2). On the contrary, chromosome 7D was preferentially inherited in the crosses involving 7DL8, 7DL4 and 7DL2 and chromosome 7Hch was preferentially inherited in the crosses involving 7DS4 and 7DL5.
The effect of specific chromosome 7D regions on lutein esterification was determined by comparing the results obtained from the different F₂ progenies. For each F₂ progeny, the half grains conserved for carotenoid analyses were pooled according to their genotype (homozygous
for 7D, class 7D7D; heterozygous, class 7D7Hch. Classes [7D7D] and [7D7Hch] were compared for carotenoid content and profile (Fig. 1).

Progenies derived from 7DS4 produced a lower proportion of esterified lutein than those derived from the other crosses (Fig. 1). Indeed, carotenoid esters accounted for 4% of total carotenoid pool in the class 7D7D from 7DS4 (Fig. 1a) while they showed at least 3-fold values in the remaining homozygous progenies. A similar behavior was observed in the heterozygous individuals (Fig. 1b). Again, the proportion of lutein esters in the heterozygous individuals derived from the cross with 7DS4 was lower than shown by the progenies derived from the other deletion lines. These results indicate that the main candidate gene for carotenoid esterification in wheat is located in the chromosome fragment missing in 7DS4. Heterozygous individuals produced higher amounts of carotenoid content than homozygous individuals for 7D due to the presence of gene Phytoene synthase1 in chromosome 7Hch (Rodríguez-Suárez et al. 2014). Besides they also produced a higher proportion of lutein esters due to the simultaneous presence of 7D and 7Hch in agreement with previous studies (Mattera et al. 2015; Mattera et al. 2017).

Deletion stocks were genotyped with DArTSeq markers. The sequences of DArTSeq markers were used as a BLASTn query against the gene models (both high confidence, HC; and low confidence, LC) available at https://wheat-urgi.versailles.inra.fr/Seq-Repository/Annotations (Alaux et al. 2018) to determine the fragment of chromosome 7D missing in each line. Markers producing a significant hit with chromosome 7D after BLASTn analyses were used to inspect the deletions of chromosome 7D in each deletion line (Table 1; Supplementary file 1). DArTSeq markers confirmed the expected deletion profile of the genetic stocks. Genetic stock 7DS4 lacked all the markers corresponding to genes located from 0 to 127.7 Mbp in the chromosome 7D (Supplementary file 1). Thus, the main gene(s) for carotenoid esterification in chromosome 7D are located in this region. Nevertheless, 7DS4-derived lines still produced carotenoid esters (Fig.
which indicate that other genes are contributing to carotenoid esterification as described in *H. chilense* (Mattera et al. 2015).

Studies on lutein esterification in several species have provided evidences of the role of acyltransferases (Lippold et al. 2012), esterase/lipase/thioesterase (Ariizumi et al. 2014), and GDSL esterases (Akoh et al. 2004) on carotenoid esterification. Mining the ‘Functional Annotation High Confidence’ gene models (https://wheat-urgi.versailles.inra.fr/Seq-Repository/Annotations) by ‘esterase’ and ‘acyltransferase’ searches revealed 30 candidate genes in the interval of 127.7 Mbp corresponding to the deletion of 7DS4 (Supplementary file 2). Out of these, 16 corresponded to GSDL esterase/lipase, 13 to acyltransferases and 1 to esterase/lipase/thioesterase.

The physical location of candidate genes is not enough to imply their potential involvement in lutein esterification. They must be expressed during grain development. Therefore the Wheat Expression Browser powered by expVIP (Borrill et al. 2016; Ramírez-González et al. 2018) was used to investigate the expression profile of the candidate genes in developing grains of wheat (Supplementary file 3). Only 10 candidate genes were expressed in at least one study targeting grain development (Fig. 2). The remaining genes were not expressed during grain development in any of the studies considered and thus they do not seem likely candidates for lutein esterification in wheat.

An acyltransferase-like protein from *Aegilops tauschii* (EMT31342.1) was proposed as a potential candidate for lutein esterification due to its similarity with *PES1* gene from Arabidopsis (Mattera et al. 2015). Using the sequence of EMT31342.1 as a tBLASTn query at NCBI returned AK449207 sequence as the best hit. This sequence corresponds to TraesCS7D01g076700 as revealed by BLASTn analysis at URGI Versailles (https://wheat-urgi.versailles.inra.fr/Tools) (Alaux et al. 2018). This gene was not expressed in any of the grain developmental stages considered at the Wheat Expression Browser (Supplementary file 3) and thus it does not seem a likely candidate for the locus in chromosome 7D.
A GDSL-like lipase was proposed as candidate gene for *Lute* (Ahmad et al. 2015). Out of ten candidate genes expressed during grain development (Fig. 2) seven corresponded to GDSL-like lipases. *Lute* was co-localized with Xgwm295 and wPt-3727 (Ahmad et al. 2015). The Wheat Genome Browser was used to determine the physical position of the genetic markers reported by (Ahmad et al. 2015) along with all the candidate genes identified in this work which were expressed in grain development (Fig. 3). Ahmad et al. (2015) designed the primers (LF5/LR5) from Contig03415 (similar to the putative rice gene LOC_Os06g05550 that was annotated as a GDSL-like lipase). The polymorphism targeted by LF5/LR5 primers co-localized with *Lute* locus and with the marker wPt-3727 in the population derived from the cross Haruhikari//Sunco/Indis.82 (Ahmad et al. 2015). Using Contig03415 as a BLASTn query at https://urgi.versailles.inra.fr/blast_iwgsc/ returned TraesCS07g094000 as the best hit (E-value 0.0, 99% identity). Furthermore, using LF5/LR5 as query for Primer BLAST at NCBI against *Triticum aestivum* (nr database) retrieved the sequence LS992100.1 in chromosome 7D which corresponds to TraesCS07g094000. Thus, this gene was referred as LF5/LR5 in Fig. 3. However, use of the sequence of LOC_Os06g05550 as a tBLASTx query against IWGSC_RefSeq_v1.0 chromosome 7D database at https://urgi.versailles.inra.fr/blast_iwgsc/ returned TraesCS07g093200 as the best hit (E-value 1e-148, 80% identity, 87% coverage). Thus, it seems that the wheat ortholog of LOC_Os06g05550 is TraesCS7D01g093200. However, LF5/LR5 primers (Ahmad et al. 2015) are targeting a polymorphism on TraesCS7D01g094000. Thus, it is not clear which is the most likely candidate for *Lute* locus. The polymorphism targeted by LF5/LR5 primers co-localized with wPt-3727 (Ahmad et al. 2015) but they are 1.39 Mbp distant (Fig. 3). Three candidate genes (TraesCS7D01g089800, TraesCS7D01G089900 and TraesCS7D01g093200) are more closely related to wPt-3727 than TraesCS7D01g094000 (Fig. 3). Considering their annotated function and their position all four genes would be likely candidates for *Lute* locus. However, only TraesCS7D01g093200 and
**TraesCS7D01g094000** were expressed in the endosperm (Fig. 2) and thus they are more likely candidates.

Ahmad *et al.* (2015) detected negligible amounts of lutein esters in the embryo. This might indicate that the gene responsible of *Lute* could be expressed in the endosperm but not in the embryo. No transcripts for **TraesCS7D01g094000** were detected in the embryo of Azhurnaya (Fig. 2) which would be in agreement with the lack of lutein esters in this tissue. However, we can only speculate since we do not know if Azhurnaya is able to synthesize lutein esters. Besides it is possible that similar but different genes are contributing to lutein esterification in different tissues.

Indeed, dissection of embryo and endosperm in tritordeum seeds revealed that both tissues are able to produce lutein esters although a greater esterifying activity was observed in the endosperm compared to the embryo (Mellado-Ortega and Hornero-Méndez 2018). These differences suggest the existence of different enzymatic systems in embryo and endosperm. The hypothesis of different genes acting in different tissues is reinforced by the differences in lutein esters production between intact grains and flours from durum wheat during storage (Mellado-Ortega and Hornero-Méndez 2017). These differences suggests that lutein esterification in flour may be produced due to the release of enzymes produced in other tissues (such as seed coat) which suggests the existence of different pathways for lutein esterification in different tissues (Mellado-Ortega and Hornero-Méndez 2016).

**Conclusions**

The results presented here demonstrate that the main gene for lutein esterification is located in the distal region of chromosome 7D corresponding to the deletion 7DS4 and equivalent to 127.7 Mb. The gene **TraesCS7D01g094000** seems the more likely candidate for the *Lute* locus but the gene **TraesCS7D01g093200** cannot be ruled out.
Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

This research was funded by Grants AGL2014-53195-R from Ministerio de Economía y Competitividad and AGL2017-85368-P from Ministerio de Ciencia, Innovación y Universidades. Both projects are cofounded by FEDER. DHM is member of CaRed Network funded by Ministerio de Economía y Competitividad (BIO2015-71703-REDT and BIO2017-90877-REDT) and participates in EUROCAROTEN COST Action (CA15136). CMA and SGA are members of FiRCMe Network, funded by Ministerio de Economía y Competitividad (AGL2016-81855-REDT).
References


Matters, MG, Cabrera, A, Hornero-Mendez, D, Atienza, SG (2015) Lutein esterification in wheat endosperm is controlled by the homoeologous group 7, and is increased by the simultaneous presence of chromosomes 7D and 7Hch from Hordeum chilense. Crop and Pasture Science 66, 912-921.
Mattera, MG, Hornero-Méndez, D, Atienza, SG (2017) Lutein ester profile in wheat and tritordeum can be modulated by temperature: Evidences for regioselectivity and fatty acid preferential of enzymes encoded by genes on chromosomes 7D and 7H(ch). *Food Chemistry* 219, 199-206.


Rodríguez-Suárez, C, Mellado-Ortega, E, Hornero-Méndez, D, Atienza, S (2014) Increase in transcript accumulation of Psy1 and e-Lcy genes in grain development is associated with differences in seed carotenoid content between durum wheat and tritordeum. *Plant Molecular Biology* 84, 659-673.


Caption Figures

Fig. 1. Carotenoid content and profile of progenies derived from the crosses between deletion stocks for chromosome 7D and common wheat- *H. chilense* chromosome substitution line [CS(7D)7Hch]. (a) Relative contribution of lutein esters to the total carotenoid pool in F$_2$ homozygous individuals for 7D (with different deletions). (b) Relative contribution of lutein esters to the total carotenoid pool in F$_2$ heterozygous individuals (7Hch7D) (with different deletions).

Fig. 2. Expression profile of candidate genes. Data were retrieved from the Wheat Expression Browser (Borrill et al. 2016; Ramírez-González et al. 2018) and shown as log2 (tpm) (transcripts per million). Only candidate genes transcribed in at least one experiment from grain development are shown.

Fig. 3. Physical location of candidate genes for lutein esterification in chromosome 7D. Candidate genes annotated as 'esterase' or 'acyltransferase' and physically mapped in the chromosome region of 127.7 Mb missing in 7DS4 are shown. Only candidate genes expressed during grain development according to the Wheat Expression browser were considered. In addition, molecular markers around the *Lute* locus with their genetic distance (cM) were also depicted (Ahmad et al. 2015).
Table 1. Genetic stocks used in this work.

1 All genetic stocks share the genetic background of Chinese Spring (CS) common wheat
2 Fraction length. It indicates the position of the breakpoint from the centromere relative to the length of the complete arm. N.a.: Not applicable.
3 WGRC: Wheat Genetic Resource Center, Kansas State University.
   JIC: John Innes Centre, UK.

<table>
<thead>
<tr>
<th>Genetic stock(^1)</th>
<th>Type</th>
<th>FL(^2)</th>
<th>Origin(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7DS-4</td>
<td>Deletion</td>
<td>0.61</td>
<td>WGRC</td>
</tr>
<tr>
<td>7DL-2</td>
<td>Deletion</td>
<td>0.61</td>
<td>WGRC</td>
</tr>
<tr>
<td>7DL-4</td>
<td>Deletion</td>
<td>0.76</td>
<td>WGRC</td>
</tr>
<tr>
<td>7DL-5</td>
<td>Deletion</td>
<td>0.30</td>
<td>WGRC</td>
</tr>
<tr>
<td>7DL-6</td>
<td>Deletion</td>
<td>0.10</td>
<td>WGRC</td>
</tr>
<tr>
<td>7DL-8</td>
<td>Deletion</td>
<td>0.77</td>
<td>WGRC</td>
</tr>
<tr>
<td>CS7Hch(7D)</td>
<td>Substitution</td>
<td>N.a.</td>
<td>JIC</td>
</tr>
</tbody>
</table>
Table 2. Segregation of chromosomes 7D and 7Hch in the F2 progenies derived from the cross between CS deletion stocks and CS disomic substitution line

<table>
<thead>
<tr>
<th>Cross</th>
<th>7Hch7Hch (%)</th>
<th>7Hch7D (%)</th>
<th>7D7D (%)</th>
<th>Goodness-of-fit(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7DL2 × CS7Hch(7D)</td>
<td>21.6</td>
<td>39.8</td>
<td>38.6</td>
<td>1.9E-06</td>
</tr>
<tr>
<td>7DL4 × CS7Hch(7D)</td>
<td>8.8</td>
<td>53.6</td>
<td>37.6</td>
<td>6.2E-11</td>
</tr>
<tr>
<td>7DL5 × CS7Hch(7D)</td>
<td>37.5</td>
<td>48.7</td>
<td>13.8</td>
<td>3.7E-07</td>
</tr>
<tr>
<td>7DL6 × CS7Hch(7D)</td>
<td>22.3</td>
<td>57.1</td>
<td>20.5</td>
<td>0.06</td>
</tr>
<tr>
<td>7DL8 × CS7Hch(7D)</td>
<td>3.1</td>
<td>31.9</td>
<td>65.0</td>
<td>4.2E-50</td>
</tr>
<tr>
<td>7DS4 × CS7Hch(7D)</td>
<td>40.2</td>
<td>50.2</td>
<td>9.7</td>
<td>3.4E-11</td>
</tr>
</tbody>
</table>