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Identification of candidate genes for lutein esterification in wheat (*Triticum aestivum* L.) using physical mapping and genomics tools

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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 F₂ 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 *Phytol 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 F₂ progenies derived from crosses between a set of deletion

lines for chromosome 7D in CS background and the CS-*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-*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).

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 °C. 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 °C 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 Mínguez-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 $\mu\text{g/ml}$. 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 $\mu\text{g/g}$ 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. F_1 hybrids were checked for chromosomes 7D and 7Hch using chromosome-specific markers before the development of F_2 progenies by selfing.

A total of 1,728 F_2 individuals (288 from each cross) were genotyped with 7D- and 7Hch-specific markers. The majority of the F_2 populations did not fit to mendelian inheritance (Table 2). This was expected since F_1 hybrids carried a deleted 7D and an alien 7Hch chromosome. Only the progeny derived from the cross 7DL6 $\times$ 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 F_2 progenies. For each F_2 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.

1) 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 GDLS-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 GDLS-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 GDLS-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.

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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 F₂ homozygous individuals for 7D (with different deletions). (b) Relative contribution of lutein esters to the total carotenoid pool in F₂ 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.

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

Genetic stock ¹	Type	FL ²	Origin ³
7DS-4	Deletion	0.61	WGRC
7DL-2	Deletion	0.61	WGRC
7DL-4	Deletion	0.76	WGRC
7DL-5	Deletion	0.30	WGRC
7DL-6	Deletion	0.10	WGRC
7DL-8	Deletion	0.77	WGRC
CS7Hch(7D)	Substitution	N.a.	JIC

421

422

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

Chi-square goodness-of-fit to Mendelian inheritance¹

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

(a)

(b)

	TraesCS7D01G004200	TraesCS7D01G011600	TraesCS7D01G037200	TraesCS7D01G089800	TraesCS7D01G089900	TraesCS7D01G093200	TraesCS7D01G094000	TraesCS7D01G108900	TraesCS7D01G109200	TraesCS7D01G111800
CS_grain, grain, whole endosperm, reproductive, 10 dpa, none, Chinese Spring, Chinese Spring(n=4)										
CS_grain, grain, whole endosperm, reproductive, 20 dpa, none, Chinese Spring, Chinese Spring(n=4)										
CS_grain, grain, starchy endosperm, reproductive, 20 dpa, none, Chinese Spring, Chinese Spring(n=4)										
CS_grain, grain, aleurone layer, reproductive, 20 dpa, none, Chinese Spring, Chinese Spring(n=6)										
CS_grain, grain, transfer cells, reproductive, 20 dpa, none, Chinese Spring, Chinese Spring(n=4)										
CS_grain, grain, starchy endosperm, reproductive, 30 dpa, none, Chinese Spring, Chinese Spring(n=4)										
CS_grain, grain, aleurone layer and starchy endosperm, reproductive, 30 dpa, none, Chinese Spring, Chinese Spring(n=4)										
12dpa_grain, grain, starchy endosperm, reproductive, 12 dpa, none, other, Holdfast(n=3)										
12dpa_grain, grain, aleurone layer, reproductive, 12 dpa, none, other, Holdfast(n=3)										
12dpa_grain, grain, seed coat, reproductive, 12 dpa, none, other, Holdfast(n=3)										
Azhurnaya_development, grain, grain, reproductive, milk grain stage, none, Azhurnaya, Azhurnaya(n=3)										
Azhurnaya_development, grain, grain, reproductive, Soft dough, none, Azhurnaya, Azhurnaya(n=3)										
Azhurnaya_development, grain, grain, reproductive, Hard dough, none, Azhurnaya, Azhurnaya(n=3)										
Azhurnaya_development, grain, endosperm, reproductive, Dough, none, Azhurnaya, Azhurnaya(n=3)										
Azhurnaya_development, grain, embryo proper, reproductive, Dough, none, Azhurnaya, Azhurnaya(n=3)										
Azhurnaya_development, grain, grain, reproductive, Ripening, none, Azhurnaya, Azhurnaya(n=3)

Chromosome 7D (from start to 68.8 Mbp)