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IDENTIFICATION OF INTROGRESSED CHROMOSOME SEGMENTS OF DURUM WHEAT IN A HIGH GRAIN WEIGHT INTROGRESSION LINE OF BREAD WHEAT cv. PBW343 THROUGH GRAPHICAL GENOTYPE ANALYSIS

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Present study provides a graphical account of introgression of chromosomal regions of durum wheat into bread wheat achievable just in two back crosses. Bread wheat cv. PBW343 was crossed with a durum wheat line DWR1006 and the F1 was backcrossed two times with cv. PBW343. In the BC_2 generation an interesting hexaploid wheat line PBW343/DWR1006 was isolated that had higher grain weight than the grain weight of both its parental genotypes. The graphical genotype analysis was carried out of an introgressed line of hexaploid wheat line PBW343/DWR1006 that has high grain weight with a view to determine the chromosomal regions inherited from the hexaploid and durum wheat parental genotypes. For this purpose, a set of 445 SSRs mapped on different chromosomes of A and B sub-genomes of wheat were tried. Out of 445 SSRs, 276 SSRs showed polymorphism between the hexploid bread wheat cv. PBW343 and the tetraploid durum wheat line DWR1006, the two parental genotypes of the hexploid introgressed line PBW343/DWR1006 and the 276 polymorphic SSRs were utilized for graphical genotype analysis of the introgressed hexaploid line PBW343/DWR1006. The number of polymorphic SSRs mapped on each of the 14 chromosomes of A and B sub-genomes varied from 11 (chromosome 4B) to 25 (chromosome 2A). It may be noted that each of the 14 A and B sub-genomes chromosomes had variable proportions of the genomic regions inherited from the hexaploid bread and tetraploid durum wheat. Each of the three chromosomes 1A, 2B and 5B had equal proportion (50%) of genome each from the hexaploid bread and tetraploid durum wheat. The chromosomes 1B and 7A had relatively large shares of regions from the hexaploid bread wheat than the tetraploid durum wheat. Each of the remaining nine chromosomes had as much as 53.84% (chromosome 3B) to 78.26% (chromosome 6B) regions from the durum wheat parental genotype DWR1006. As expected the overall chromosomal regions contributed by the durum wheat was higher (56.80%) than the regions contributed by the hexaploid wheat (43.17%).

Keywords: Durum wheat, Bread wheat, BC2, Graphical genotype analysis, introgression

Wheat belongs to genus *Triticum* of tribe Triticeae of the family Poaceae and comprises diploid (2n=2x=14), tetraploid (2n=4x=28) and hexaploid (2n=6x=42) species. The hexaploid wheat (*Triticum aestivum* L.), which is often described as 'bread wheat' or 'common wheat' is cultivated over a wide range of climatic and soil conditions. In India, wheat is cultivated on an area of about 29.8 m ha with a production of 92.3 mt in 2012-13, which contributes approximately 13.53% to the world's total wheat production (FAO 2013, http://www.icar.org.in/news/record-production-foodgrain.htm). Although wheat production in India has increased 14 times to a record 92.3 mt in 2012-13 from 6.5 mt in 1950, it still remains inadequate to feed the fast growing population of India.

During the course of this investigation hexaploid wheat line with grain weight higher than both its parental genotype has been isolated. Since grain weight is a quantitative trait, its genetic dissection is a very challenging task. As a first step in this direction, we decided to identify the chromosomal segment that have been introgressed into the above high grain weight hexapod line from the durum wheat. In order to achieve this, the present study employed graphical genotype analysis using a large number of SSR markers that are mapped on the chromosomes of both A and B sub-genomes of wheat (Somers et al. 2004).

MATERIALS AND METHODS

Seed Material

Present study utilized three wheat genotypes. These included a bread wheat cv. PBW343 (*Triticum aestivum* L.) and durum wheat (*Triticum durum* Desf.) genotype DWR1006 and a high grain weight bread wheat (*Triticum aestivum* L.) line (PBW343/DWR1006) selected in the BC_2 generation derived from the backcrossing of the F1 hybrid PBW343/DWR1006 two times with PBW343. The seed

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material was obtained from DWR, Karnal, Haryana.

SSR Markers

A set of 445 SSRs comprising 235 wmc, 129 gwm, 8 cfd, 7 gdm, 14 cfa, and 52 barc markers which are specific to A and B sub-genomes of wheat were selected from the wheat molecular map constructed by Somers et al. (2004). The details of the SSRs used are given in Table 1. The selected markers were used for detection of polymorphism between the two parental genotypes (PBW343 and DWR1006) and the derived line with high grain weight.

Field experiment and recording of data

The three wheat genotypes, namely PBW343, DWR1006 and the high grain weight bread wheat line (PBW343/DWR1006) were raised in three row plots at Research Farm, Department of Genetics and Plant Breeding,

Table 1. List of the SSRs belonging to the A and B sub-genome chromosomes of wheat used during the present study

Chromosome number	Number of SSR primers	Name of SSR primers
1A	22	gdm33, gwm136, gwm33, wmc95, gdm136, wmc24, gwm357, barc119, gwm164, cfd30, gwm135, wmc278, wmc611, wmc469, wmc183, wmc312, cfa2129, wmc9, wmc673, wmc59, barc145, gwm99.
1B	21	gwm608, gwm550, wmc406, barc8, gwm33, gwm494, gwm131, cfd2, gdm136, wmc216, cfa2129, cfd48, wmc416, gwm403, wmc206, gwm153, wmc766, gwm124, wmc719, wmc367, wmc728.
2A	25	gwm296, wmc667, gwm497, wmc407, gwm359, wmc177, wmc598, wmc149, wmc453, wmc522, wmc474, gwm122, gwm275, gwm249, gwm558, gwm473, gwm328, gwm372, wmc261, gwm294, wmc181, gwm356, barc76, gwm382, gwm311
2B	24	wmc764, barc124, wmc154, wmc243, barc10, gwm410, wmc272, wmc474, wmc344, gwm374, gwm403, wmc498, gwm271, gwm129, gwm388, wmc51, gwm120, gwm47, wmc332, wmc149, wmc317, barc159, gwm382, wmc356
3A	24	wmc11, wmc532, gwm369, gwm5, gwm30, cfd193, gwm403, cfa2134, cfa2234, wmc428, wmc264, cfa2262, gwm494, wmc96, gwm497, c fa2193, wmc17, cfd2, gwm155, wmc153, cfa2076, wmc169, wmc594, gwm480.
3B	13	wmc540, gwm566, gwm284, gwm107, wmc471, wmc418, cfa2134, gwm131, gwm4, barc206, wmc326, gwm547, gwm247.
4A	22	gwm165, wmc48, wmc446, gwm44, gwm397, wmc513, wmc650, barc170, wmc468, wmc258, wmc161, gwm494, wmc760, wmc283, wmc262, wmc698, wmc500, wmc232, cfd30, cfd2, wmc497, wmc219
4B	11	wmc125, wmc47, barc68, barc163, gwm251, cfd39, gwm149, gwm193, gwm112, wmc419, cfd2.
5A	22	barc10, wmc713, wmc489, gwm293, gwm129, gwm304, wmc4 46, cfa2121, wmc492, gwm639, wmc415, cfd2, gwm666, wmc445, cfa2163, cfa2141, wmc96, cfa2185, wmc110, gwm179, wmc524, gwm410.

5B	16	wmc773, wmc47, wmc728, wmc149, wmc274, gwm133, gwm191, wmc386, barc109, wmc363, gwm274, wmc405, wmc537, gwm271, gwm604, wmc508.
6A	19	gwm459, barc206, barc23, wmc182, barc37, barc48, wmc672, wmc145, wmc256, wmc684, gwm132, wmc553, wmc179, wmc417, wmc580, wmc206, wmc59.
6B	23	wmc419, wmc486, barc76, wmc487, gwm132, cfd1, gdm113, wmc494, gwm191, gwm193, gwm133, gwm644, wmc397, wmc473, wmc182, gwm311, wmc748, wmc539, gwm626, wmc152, gwm107, barc178, barc134.
7A	22	gwm666, wmc158, wmc388, wmc497, wmc646, gwm471, wmc168, gwm60, wmc593, wmc179, wmc283, barc154, wmc83, wmc405, gwm260, wmc17, wmc182, wmc596, barc23, cfd193, gwm63, wmc525.
7B	14	wmc606, barc72, wmc758, gwm297, gwm644, wmc364, wmc396, gwm213, gwm274, gwm131, wmc517, wmc10, wmc500, barc123.

CCS University, Meerut. The grains of each of the three genotypes were harvested separately in bulk. Ten random samples each of 100 harvested grains per genotype were separately drawn and weighed using an electronic balance.

Extraction, purification and estimation of concentration of DNA

(a) DNA extraction: Genomic DNA from leaves of one-month old field grown plants was isolated using CTAB (cetyltrimethyl ammonium bromide) method of Saghai-Maroof *etal.* (1984), which involved the following steps: (i) Approximately 3 g leaf material was ground to fine powder in liquid nitrogen with the help of mortar and pestle. The fine powder was immediately transferred to 15 ml of pre-warmed (60ºC) isolation buffer in a 50 ml capped polypropylene centrifuge tube and incubated for about 1h at 60 ºC in water bath and mixed by gentle swirling after every 10 min. (ii) To each of the above tubes, equal volume of chloroform (C): isoamyl alcohol (I) (24:1) was added and the tubes were gently shaken for 10 min. (iii) The above tubes were centrifuged for 10 min at 5000 rpm at room temperature and the upper aqueous phase was transferred to a fresh sterile 50 ml centrifuge tube. (iv) To the above tubes 0.6 volume of ice cold isopropanol was added and after shaking, the tubes were kept at -20 ºC for 1 h. (v) With the help of sterile glass hook, DNA was spooled out and transferred to 5 ml of washing solution (70% ethanol) for 10 min at room temperature. (vi) DNA on the hook was air-dried for 1 h. (vii) The air-dried DNA was dissolved in 3 ml of 1X TE buffer.

(b) DNA purification: Purification of the DNA extracted as above involved the following steps (Sambrook etal., 1989): (i) For purification, 30 µg RNase A was added to the tube, and the mixture was incubated at 37°C (25:24:1) by centrifuging the tubes at 10000 rpm for 5 min at room temperature and re-extracted with CI by centrifuging the tubes again at 10000 rpm for 5 min at room temperature. (ii) The aqueous phase from the above tube was added to a 15 ml tube, and twice the volume of ice-cold absolute alcohol was added to precipitate the genomic DNA. (iii) The DNA was spooled out with the help of sterile glass hook and was washed in 70% ethanol, air-dried and dissolved in 500 µl of 1X TE buffer in an eppendorf tube.

(c) Estimation of the concentration of DNA: For the concentration of the above purified genomic DNA, the ratio of absorbance at 260 nm and 280 nm on spectrophotometer was used to assess the purity of DNA and by the known

amount of DNA on an agarose gel.

PCR analysis

(a) PCR amplification: The PCR amplification of genomic DNA using different SSR primers was carried out as given by Roder *etal.* (1998). The composition of PCR reaction mixture and PCR cycling profiles for amplification of SSRs are given in Table 2. The amplified products due to each primer pairs were resolved separately following polyacrylamide gel electrophoresis (PAGE) and visualized following silver staining.

(b) PAGE: The amplified product after mixing with the loading buffer/dye was subjected to electrophoresis at constant voltage of 200-250V for 6-8 h in MEGA-GEL High Throughput Vertical Unit of C.B.S Scientific Co. (Wang *etal.* 2003). After electrophoresis, the gels were stained using silver staining.

(c) Silver staining: To resolve the amplified products due to SSRs, silver staining was carried out following Tagelstrom (1992) with some modifications. Following steps were involved: (i) After electrophoresis, the gel was carefully removed from the glass plates and transferred to a tray containing double distilled water and kept for 5 min with gentle shaking. (ii) The distilled water in the above tray was then replaced with fixing solution containing 10% ethanol/methanol and 0.50% glacial acetic acid and kept for another 5 min with gentle shaking. (iii) The above fixing solution was removed from the tray and retained for further use. Silver solution (0.3g $AgNO_3$ powder in 150 ml 10% ethanol/methanol solution with 750 µl glacial acetic acid) was then poured in the tray for staining the gels, The gels was kept in the silver solution for 5 min with gentle shaking. (iv) The silver solution was removed from the tray and the gel was rinsed for a while in distilled water. (v) The gel was transferred to the developing solution (prepared by dissolving 4.0 g NaOH pellets in 150 ml distilled water with 450 µl of 40%

Table 2. Details of the PCR reaction mixture and the cycling profiles used in PCR amplification reactions.

Reaction mixture	**Volume (µl)**
Total reaction volume (µl)	20
Template DNA (ng)	2
Primer (µm)	5
dNTP (mM)	0.5
$MgCl_2$ (mM)	1.0
10X PCR Buffer	2
Taq polymerase (U)	0.1
Double distilled water up to	9.4
PCR profile	Time
No. of cycles	45
Initial denaturation (º C)	95/5 min
Denaturation (º C)	95/5 min
Annealing	50/55/60/61 1 min
Extension (º C)	72/1 min
Ramp	0.5 ºC/s
Final extension (º C)	72/10 min
Soak temperature (º C)	4 min

*Annealing temperature was lowered by 1ºC per cycle during the first 10 cycles, and then remaining 23 cycles were performed at 56ºC annealing temperature.

formaldehyde). (vi) The solution in the tray was shacked gently for 5-10 min allowing the DNA bands to appear. (vii) The staining was stopped by rinsing the gel for 5 min in the fixing solution retained after step (iii).

Recording of data

The silver stained gels were placed on a light box and photographed using digital camera and the picture was transferred for recording of data. For each SSR, the data was recorded in 1/0 format. Code 1 was given to the SSR allele of the bread wheat cultivar (PBW343) and 0 was given to allele of the durum wheat cultivar (DWR1006). In case of the introgression hexaploid bread wheat line with high grain weight, 1 or 0 was given to the alleles, respectively, corresponding to the bread wheat and the durum wheat.

Statistical analysis

(a) t-test: The significance of difference between the mean grain weight of the two samples was tested using software SPSS.

(b) Graphical genotype analysis: Graphical genotype analysis that enables representation of molecular marker data by simple chromosome drawings was done using GGT2.0 Software (http:// www. plantbreeding.wur.nl/uk/software_ggt.html).

RESULTS AND DISCUSSION

An interesting hexaploid wheat line PBW343/DWR1006 obtained from a bread wheat cv. PBW343 crossed with a durum wheat line DWR1006; and the F1 generation backcrossed twice with cv. PBW343 had higher grain weight than the grain weight of both its parental genotypes (Fig. 1). The developed introgression line exhibiting more than additive type of epistasis for grain weight has been chosen to help understand the genetic control of grain weight in order to designing suitable breeding approaches for the transfer of high grain weight. As a first step towards understanding the genetic control of high grain weight in the introgression line, its graphical genotype analysis was undertaken using SSR markers during the present study.

Grain Weight of Parental Genotypes and the Introgression Line

The 1000-grain weight (52.90g) of the introgression line PBW343/DWR1006 was, significantly higher than the grain weight of both its parental genotypes, i.e. the tetraploid durum wheat genotype DWR1006 (47.35g) and the hexaploid bread wheat cv. PBW343 (42.78g) (Fig. 1), suggesting more than additive type of epistatic interaction for grain weight (elaborate it).

Graphical Genotype Analysis

The graphical genotype analysis was carried out of an introgressed line of hexaploid wheat line PBW343/DWR1006 that had high grain weight with a view to determine the chromosomal regions inherited from the hexaploid and durum wheat parental genotypes. For this purpose, a set of 445 SSRs mapped on different chromosomes of A and B sub-genomes of wheat (Somers et al. 2004)

Figure 1. Digital images of grain size of two parental genotypes (A = PBW343 and B = DWR1006) and the introgression line (C = PBW343/DWR1006)

were tried. Out of 445 SSRs, 276 SSRs showed polymorphism between the hexploid bread wheat cv. PBW343 and the tetraploid durum wheat line DWR1006, the two parental genotypes of the hexploid introgressed line PBW343/DWR1006 and the 276 polymorphic SSRs were utilized for graphical genotype analysis of the introgressed hexaploid line PBW343/DWR1006 (Fig. 2). The number of polymorphic SSRs mapped on each of the 14 chromosomes of A and B sub-genomes varied from 11 (chromosome 4B) to 25 (chromosome 2A).

The details of the graphical genotype analysis are presented in Table 3 and Fig. 3a, b. It may be noted that each of the 14 A and B sub-genome chromosomes had variable proportions of the genomic regions inherited from the hexaploid bread and tetraploid durum wheat. Each of the three chromosomes 1A, 2B and 5B had equal proportion (50%) of genome each from the hexaploid bread and tetraploid durum wheat. The chromosomes 1B and 7A had relatively large shares of regions from the hexaploid bread wheat than the tetraploid durum wheat. Each of the remaining nine chromosomes had as much as 53.84% (chromosome 3B) to 78.26% (chromosome 6B) regions from the durum wheat parental genotype DWR1006. As expected the overall chromosomal regions contributed by the durum wheat was higher (56.80%) than the regions contributed by the hexaploid wheat (43.17%).

Chromosomal Regions with QTL for GW

A large number of QTL for GW have been mapped in both the hexaploid bread and tetraploid durum wheat in the past (see Table 1 and 2). Previously reported location of QTL for GW in chromosomal regions inherited from the hexaploid bread wheat and tetraploid durum wheat in the introgressed line with high GW are shown in Fig. 3a,b. These QTL may be involved in determining the high GW of the introgression line. Due to the large number of chromosomal regions from tetraploid durum wheat (earlier reported to carry QTL for GW) in the introgression line, it is not possible at the moment to identify the specific chromosomal regions that may be involved more than additive type of epistatic interaction in determining the high GW of the introgression line. In the present investigation we have successfully isolated the introgression line PBW343/DWR1006 with

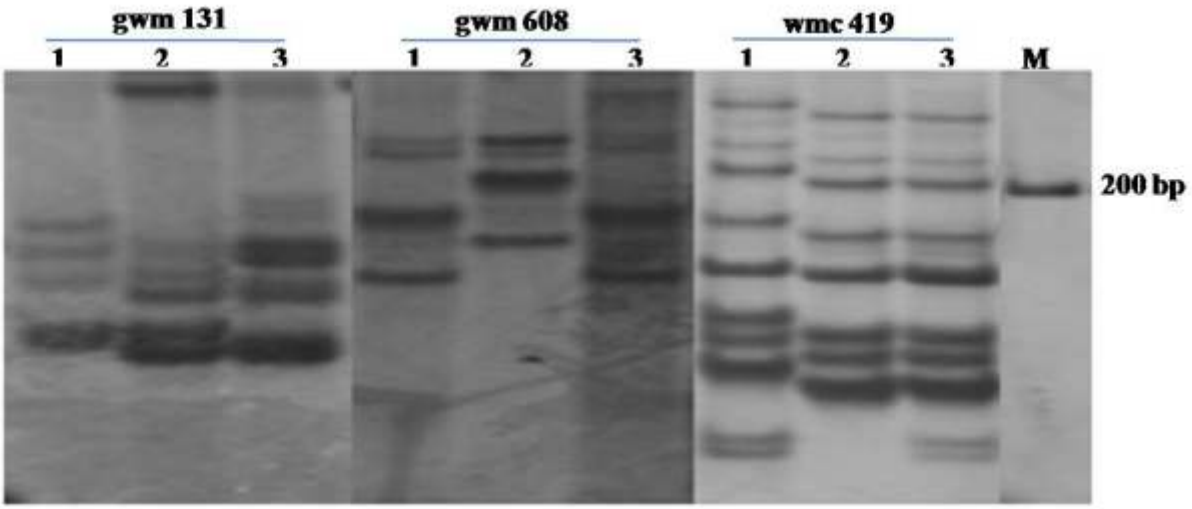

Figure 2. A representative sample of amplification profiles of two parental genotypes (1=PBW343 and 2=DWR1006) and introgression line (3=PBW343/DWR1006). M = 100 bp DNA ladder. The amplification profile of the introgression line (PBW343/DWR1006) due to the SSR marker gwm131 and wmc419 is similar to the profile of DWR1006, tetraploid wheat. The amplification profile of the introgression line (PBW343/DWR1006) due to SSR marker gwm608 is similar to that of PBW 343, hexaploid wheat.

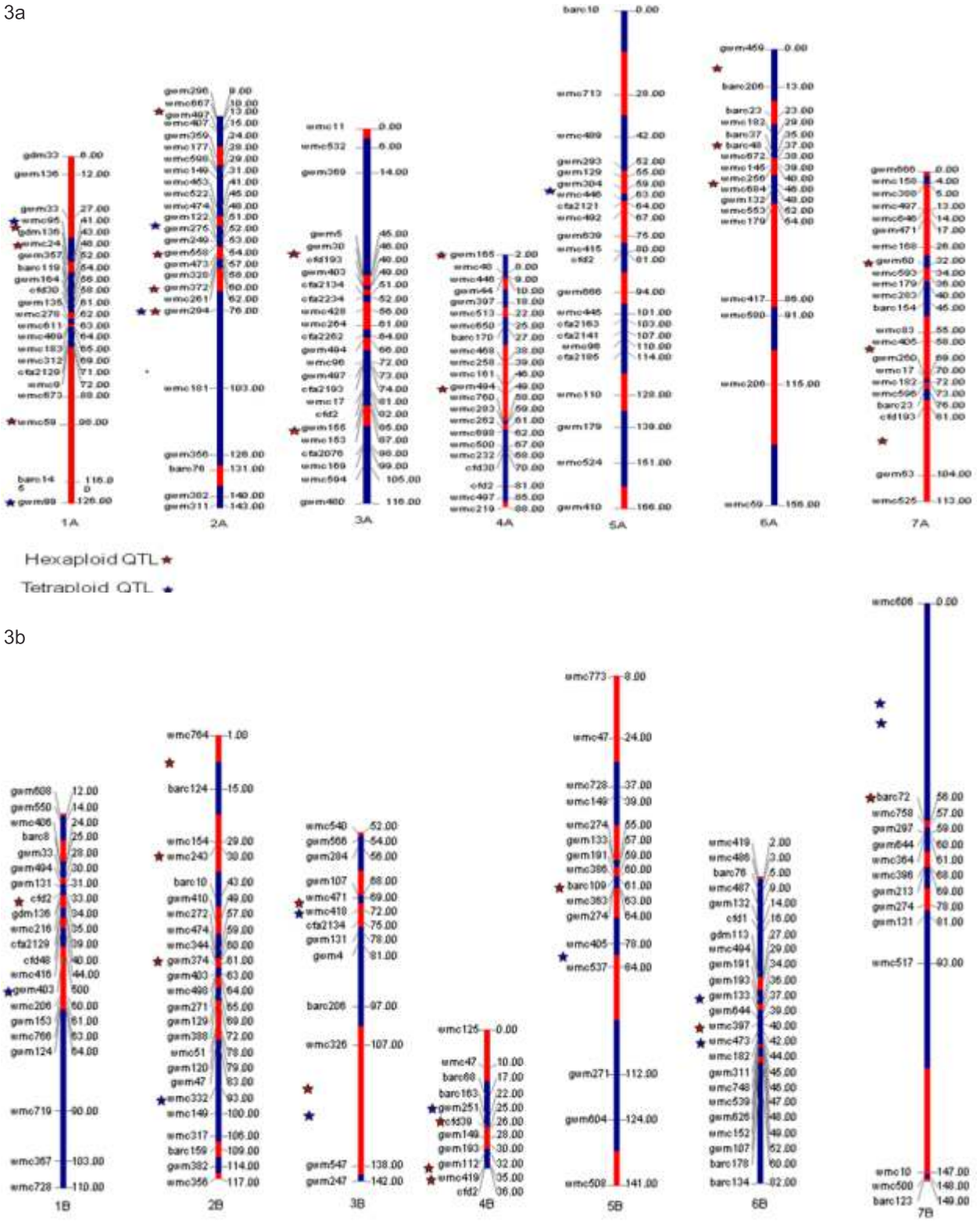

Figure 3a, b. Graphical genotypes of chromosomes of high grain weight introgression wheat lines PBW343/ DWR1006 drawn by the GGT2.0 where (a) seven chromosomes belonging to A sub-genome (1A to 7A), (b) seven chromosomes belonging to B sub-genome (1B to 7B). Marker locations were based on the map positions from the Somers et al. (2004). The hexaploid wheat genome is shown in red colour, and the tetraploid genome is shown in blue colour. The red stars indicate QTL mapped in the hexaploid wheat and the blue stars indicate QTL mapped in tetraploid wheat.

Table 5. Percent chromosome segments of A and B sub-genomes belonging to the bread and durum wheat genotypes in the high grain weight introgression line of bread wheat. The number of chromosome segments belonging to the bread wheat and durum wheat genotypes in each of the 14 chromosomes is given in parenthesis.

S. No.	Chromosome	No. of SSRs used	Percent chromosome segments inherited from the two parental genotypes	
			Hexaploid bread Wheat	Tetraploid durum wheat
1.	1A	22	50.00 (5)	50.00 (4)
2.	1B	21	52.38 (6)	47.61 (6)
3.	2A	25	24.00 (5)	76.00 (6)
4.	2B	24	50.00 (8)	50.00 (7)
5.	3A	24	33.33 (6)	66.66 (6)
6.	3B	13	46.15 (4)	53.84 (4)
7.	4A	22	40.90 (5)	59.09 (5)
8.	4B	11	45.45 (2)	54.54 (2)
9.	5A	22	40.90 (6)	59.09 (6)
10.	5B	16	50.00 (6)	50.00 (5)
11.	6A	17	41.17 (4)	58.82 (5)
12.	6B	23	21.73 (5)	78.26 (5)
13.	7A	22	72.72 (6)	27.27 (5)
14.	7B	14	35.71 (4)	64.28 (4)
Average	-	-	43.17	56.80

higher grain weight containing 58.60% chromosomal regions from durum wheat and 43.17 % chromosomal regions from hexaploid wheat due to large number of introgressed chromosomal regions tetraploid wheat. We unable or failed to pinpoint the specific regions responsible for higher grain weight.

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