African Journal of Biotechnology Vol. 11(58), pp. 12167-12172, 19 July, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.677
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Detection of the introgression loci in *Triticum aestivum* transferred from *Aegilops tauschii* by simple sequence repeat (SSR) markers

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Accepted 25 April, 2012

Aegilops tauschii **possesses many valuable traits or genes. The introgression loci transferred from** ***A. tauschii*** **in populations of 64 lines of BC_1F_2 and 147 advanced lines of BC_1F_2-F_7 derived from the cross of Chinese Spring (CS;** ***Triticum aestivum*****) /SQ-214 (***A. tauschii)* **//SW3243 (***T. aestivum***) was characterized using simple sequence repeat (SSR) markers. 68 polymorphic loci covering the D genome were used to analyze the distribution of** ***A. tauschii*** **SQ-214 introgression loci in the two derived populations. The result shows that 82.35% loci of** ***A. tauschii*** **SQ-214 were detected in BC_1F_2 population, while the frequency of introgression loci was 51.47% in the advanced lines of BC_1F_2-F_7 after artificial selection for stripe rust resistance and agronomic traits. Significant changes of the distribution frequency for the same introgression loci between BC_1F_2 and BC_1F_2-F_7 populations were observed, and seven selective advantage loci from** ***A. tauschii*** **(Xgdm128-3D, Xgdm8-3D, Xgdm72-3D, Xgwm341-3D, Xgdm63-5D, Xgdm132-6D and Xgdm36-6D) were found, which introgression loci distribution frequency increased from 29.69 to 53.91% (BC_1F_2) to 94.22 to 100% (BC_1F_2-F_7). The selective advantage loci could be used to evaluate the desirable genes or traits derived from** ***A. tauschii*** **SQ-214.**

Key words: *Aegilops tauschii*, *Triticum aestivum*, introgression locus.

INTRODUCTION

Aegilops tauschii Coss. (DD, 2n = 14) is a wild diploid species and the D genome donor to *Triticum aestivum* (AABBDD, 2n = 42), and possess a lot of desirable genes for wheat breeding, such as pest resistance (Zhu et al., 2005), stem rust resistance (Mattew et al., 2011; Assefa and Fehrmann, 2004), stripe rust resistance (Yang et al., 2003; Liu et al., 2010) and other valuable genes (Friesen et al., 2008). The genetic diversity in D genome of *A. tauschii* was richer than the D-genome of *T. aestivum* (Naghavi et al., 2009; Giles and Brown, 2006). Therefore, *A. tauschii* has been used extensively for the transfer of elite genes to wheat.

Introgression has played a crucial role in enriching genetic diversity and germplasm development (He et al., 2005; Liu et al., 2006; Zhang et al., 2005). Introgression loci from crop relatives can be easily detected by molecular markers. Among various markers, simple sequence repeat (SSR) marker is efficient tool due to a high level of polymorphism and chromosome specificity. Frequency of alleles and DNA segment linked to desirable genes can increase significantly under positive selection. Knowledge on the distribution of introgression loci will help us to target desirable genes. Zhang et al. (2005) analyzed the introgression frequency of synthetics donor alleles by SSR, and found some selective advantage alleles linked to desirable traits or genes.

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Abbreviations: CS, Chinese Spring, **SSR**, simple sequence repeat.

SQ-214 was selected from 45 accessions of *A. tauschii* introduced from CIMMYT, it was highly resistance to three Chinese stripe rust physiological stains CYR30, CYR31 and CYR32, and possesses good quality subunit (Lu et al., 2005; Yang et al., 2003). Genetic analyses revealed that the resistance of SQ-214 was control by a single dominant gene. To exploit the resistance to stripe rust and agronomic traits in *A. tauschii* SQ-214, it was used as resources for elite genes transferring, and two sets of populations of BC_1F_2 and BC_1F_2-F_7 lines derived from the combination of CS/SQ-214//SW3243 were developed.

The objectives of this study were to: (i) detect the introgression loci from *A. auschii* SQ-214 in its derived wheat populations, (ii) compare the introgression loci of *A. tauschii* SQ-214 on different chromosomes in its derivative populations, and (iii) analyze selective advantage loci.

MATERIALS AND METHODS

Plant materials

A. tauschii SQ-214, wheat landrace Chinese Spring (CS), a commercial wheat variety SW3243, 64 lines of BC_1F_2 and 147 lines of BC_1F_2-F_7 derived from the combination of CS/SQ-214 //SW3243 were used in this study. The 64 individual lines were selected randomly from the BC_1F_2 population, and 147 ones with better agronomic character and high resistance were selected from BC_1F_2 to BC_1F_2-F_7. The three parents (CS, SQ-214 and SW3243) were used as reference materials.

SSR analyses

Genomic DNA was isolated from fresh leaves of a single seedling by the cetyltrimethylammonium (CTAB) method of Röder et al. (1998). Quality and quantity of the isolated DNA was determined on 1% (w/v) agarose gels by comparing bands to know concentrations of DNA.

118 SSR specific primers detecting polymorphism among CS, SQ-214, and SW3243 on D genome of wheat were selected from the wheat microsatellite maps of Röder et al. (1998), Pestsova et al. (2000) and Somers et al. (2004). These primers covered the seven different chromosomes of D genome, with at least four loci on each chromosome.

Polymerase chain reaction (PCR) reactions were performed in a volume of 25 µl in a model PTC100 thermocycler. The reaction mixture (Röder et al., 1998) contained 10 × PCR buffers, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 250 nM of each primer, 50 to 100 ng template DNA, and 1U *Taq*-polymerase. PCR was performed with the following program: After 3 min denaturing at 94°C, 45 cycles consisting of 1 min denaturation at 94°C, 1 min annealing (temperature depending on the different primer combinations) and 2 min extension at 72°C was observed and a final extension step of 10 min at 72°C. PCR products were separated using 6% polyacrylamide denaturing gels (Zhu et al., 2004). Before loading sample, the PCR products were mixed with an equal amount of buffer. The gels were run in of 1 × Tris-borate-EDTA (TBE) buffer with 400 V. After electrophoresis, bands were visualized using a silver staining procedure described by Bassam et al. (1991). The gel was removed from the glass plates after electrophoresis and washed three times, and then put it into about 200 mL solution of 0.1% $AgNO_3$ solution for 15 min. The stained gel was washed three times, then, placed into about 200mL solution containing 1.5% NaOH solution, 0.4% formaldehyde and 0.019% sodium borate until appearing visible DNA bands. Then the gel was covered with cellophane and photographs were taken using a digital camera.

Statistical analyses

The amplified SSR DNA bands representing different alleles were scored as different genotypes. Banding patterns of SQ-214, CS and SW3243 were used as reference to help score different alleles in the BC_1F_2 and BC_1F_2-F_7 population of CS/SQ-214//SW3243. Banding patterns of homozygote were scored as AA, BB or CC, and heterozygote genotypes were scored as AB or BC or AC.

To reveal the artificial selection effects, the frequency (F) and the distribution frequency (DF) of *A. tauschii* SQ-214 introgression loci in BC_1F_2 and BC_1F_2-F_7 populations of CS/SQ-214 //SW3243, were calculated.

$$F = n_i / N_i \times 100\% \quad DF = p_i / P_i \times 100\%$$

Where, n_i refers to the number of *A. tauschii* SQ-214 introgression loci, N_i is the number of detected loci, p_i is the number of plants with introgression locus, and P_i is the number of plants studied. Selective advantage of alleles was evaluated for each marker based on the DF value.

The framework map of *A. tauschii SQ-214* introgression loci was constructed using MapDraw V2.1 (Liu and Meng, 2003).Genetic distance was taken from Röder et al. (1998), Pestsova et al. (2000) and Somers et al. (2004).

RESULTS

Introgression loci in BC_1F_2 and BC_1F_2-F_7

118 SSR specific primers in the D genome of wheat were selected to detect the polymorphism among the three parents (SQ-214, CS and SW3243). 68 SSR primers with polymorphism were selected to detect the distribution of SQ-214 introgression loci in its derived wheat BC_1F_2 and BC_1F_2-F_7 populations. Results showed that 82.35% loci of *A. tauschii* (56 introgression loci) were found in BC_1F_2, and 51.47% loci (35 introgression loci) in BC_1F_2-F_7 population. About 30.88% introgression loci (21 loci) were discarded (Table 1) in the BC_1F_2-F_7 population after artificial selection.

Introgression loci on different chromosomes

The distribution of introgression loci on the seven different chromosomes of D genome was diverse as indicated by F value (frequency of *A. tauschii* introgression loci) (Table 1). In BC_1F_2 population, the distribution of introgression loci was unbalanced, and the order of F value was: 6D (100.00%) > 2D (92.31%) > 3D (90.91%) > 4D (85.71%) > 7D (80.00%) > 1D (72.73%) > 5D (66.67%). The highest F was 100%, on 6D; the lowest one was only 66.67%, on 5D. In BC_1F_2-F_7 population, the distribution of introgression loci was also unbalanced, and their F value was: 3D (72.73%) > 5D (66.67%) > 1D (63.64%) > 6D (50.00%) > 2D (38.46%) > 7D (30.00%) >

Table 1. Number and frequency of introgression loci of *A. tauschii* in BC_1F_2 and BC_1F_2-F_7.

Chromosome	No. of detected locus	Introgression locus in BC_1F_2		Introgression locus in BC_1F_2-F_7		Discarded locus in BC_1F_2-F_7	
		No. of locus	F (%)	No. of locus	F(%)	No. of locus	F(%)
1D	11	8	72.73	7	63.64	1	9.09
2D	13	12	92.31	5	38.46	7	53.85
3D	11	10	90.91	8	72.73	2	18.18
4D	7	6	85.71	2	28.57	4	57.14
5D	12	8	66.67	8	66.67	0	0.00
6D	4	4	100.00	2	50.00	2	50.00
7D	10	8	80.00	3	30.00	5	50.00
Total	68	56	82.35	35	51.47	21	30.88

F, Frequency of *A. tauschii* SQ-214 introgression loci.

Table 2. Distribution frequency change of *A. tauschii* introgression loci on 1 to 7D chromosomes.

Distribution frequency change of introgression locus		Total	1D	2D	3D	4D	5D	6D	7D
Reduced[1]	No. of locus	39	6	11	4	4	5	0	7
	F (%)	57.35	54.55	84.62	36.36	57.14	41.67	0.00	70.00
Constant[2]	No. of locus	14	3	1	1	1	4	2	2
	F (%)	20.59	27.27	7.69	9.09	14.29	33.33	50.00	20.00
Increased[1]	No. of locus	15	2	1	6	2	3	2	1
	F (%)	22.06	18.18	7.69	54.55	28.57	25.00	50.00	10.00

[1]Changes of the mean distribution frequency of *A. tauschii* introgression loci on the same locus. [2]Including the locus were constant in both of BC_1F_2 and BC_1F_2-F_7 population and not detected in the in the original BC_1F_2 population.

4D (28.57%).

The distribution of discarded loci was found on almost all of the chromosomes (except chromosome 5D) and also unbalanced. The all introgression loci on 5D in BC_1F_2 population still retained in BC_1F_2-F_7 population, but introgression loci on other chromosomes were discarded either more or less. More loci were discarded on 2, 4 and 7D with the frequency of discarded loci 53.85, 57.14 and 50.00%, respectively.

Selective advantage of introgression loci

Significantly, changes of DF for introgression loci were observed between BC_1F_2 and BC_1F_2-F_7 populations (Table 2). The DF of 79.41% introgression loci was changed between BC_1F_2 and BC_1F_2-F_7 populations. Of 79.41% changed introgression loci, 22.06% loci significantly increased, and 57.35% loci reduced significantly (Table 2). The DF value of the most introgression loci on 1, 2, 4 and 7D was reduced, while that of on 3 and 6D was increased (Table 2).

Table 3. List of locus of the DF changed significantly between BC_1F_2 and BC_1F_2-F_7 population.

Primer	Distribution frequency in BC_1F_2 (%)	Distribution frequency in BC_1F_2-F_7 (%)	Distribution frequency change (±%)
Xgdm35-2D	32.81	0.68	-32.13
Xgwm261-2D	32.03	0.00	-32.03
Xwmc111-2D	29.69	0.00	-29.69
Xwmc470-2D	26.56	0.00	-26.56
Xwmc144-2D	10.94	51.02	40.08
Xwmc492-3D	57.81	0.68	-57.13
Xgwm161-3D	38.28	69.05	30.77
Xgdm128-3D	53.91	100.00	46.09
Xgdm72-3D	43.75	98.98	55.23
Xgdm8-3D	41.41	100.00	58.59
Xgwm341-3D	37.5	96.6	59.10
Xwmc552-3D	11.72	0.00	-11.72
Xgdm38-3D	14.06	4.76	-9.30
Xgwm664-3D	3.13	0.00	-3.13
Xgwm314-3D	4.08	6.12	2.04
Xgdm61-4D	32.81	0.00	-32.81
Xwmc285-4D	25.78	78.23	52.45
Xgwm272-5D	10.16	70.07	59.91
Xgdm63-5D	31.25	94.22	62.97
Xgdm132-6D	53.13	100.00	46.88
Xgdm36-6D	29.69	99.32	69.63
Xgdm150-7D	32.81	0.00	-32.81
Xwmc42-7D	32.03	0.00	-32.03

Distribution frequency change = distribution frequency of introgression loci in advanced lines on certain locus - distribution frequency of introgression loci in BC_1F_2 on the same locus. All of the 3D loci is listed.

The DF of individual introgression loci on same chromosomes was significantly different (Table 3 and Figure 1). As a sample, the DF of each introgression loci of SQ-214 on 3D is shown in Figure 1. The distribution frequency of locus Xgdm128, Xgdm8, Xgwm161, Xgdm72 and Xgwm341 on 3D was significantly increased, while that of Xwmc492, Xgwm664 and wmc552 was almost completely discarded in BC_1F_2-F_7.

There were seven loci (Xgdm128-3D, Xgdm8-3D, Xgdm72-3D, Xgwm341-3D, Xgdm63-5D, Xgdm132-6D and Xgdm36-6D) which DF was increased from 29.69 to 53.91% in BC_1F_2 to 94.22 to 100% in BC_1F_2-F_7 (Table 3). These seven loci deviating from the expected frequency significantly should be selective advantage alleles named by previous authors (Zhang et al., 2005). Of the four selective advantage loci in 3D, three of them constructed one chromosomal region covering 9.1 cM (xgdm72-xgdm8-xgdm128) (Figure 1).

DISCUSSION

Wheat is one of the most important and wide cultivated crops in the world. To meet the needs of growing population and economic development, the demand for wheat yield and quality is expected to grow faster. *A. tauschii* is the wheat wild relative species and possesses many valuable genes for wheat improvement. Since the D genome of *A. tauschii* showed a high homology with the D genome in wheat, the elite genes of *A. tauschii* can be introgressed into wheat only through recombination of the homologous chromosomes and the undesirable gene linkages can be broken using cultivated wheat as repeated backcrossing parent (Mujeeb-Kazi et al., 1996; Valkoun, 2001). Therefore, *A. tauschii* was con-sidered as one of the most suitable germplasm among wild relatives of wheat (Assefa and Fehrmann, 2004; Mujeeb-Kazi et al., 1996; Zhang et al., 2005).

In the present study, we characterized the alleles from *A. tauschii* SQ-214 present in its derived BC_1F_2 and BC_1F_2-F_7 populations. Our results showed that 82.35% loci were transferred into wheat genetic background by crossing and backcrossing in random BC_1F_2 population, and 51.47% loci were held in BC_1F_2-F_7 advanced lines with high resistances to stripe rust and good agronomic traits after artificial selection. Significant changes of the distribution frequencies of introgression loci from *A. tauschii* were observed by comparing the original

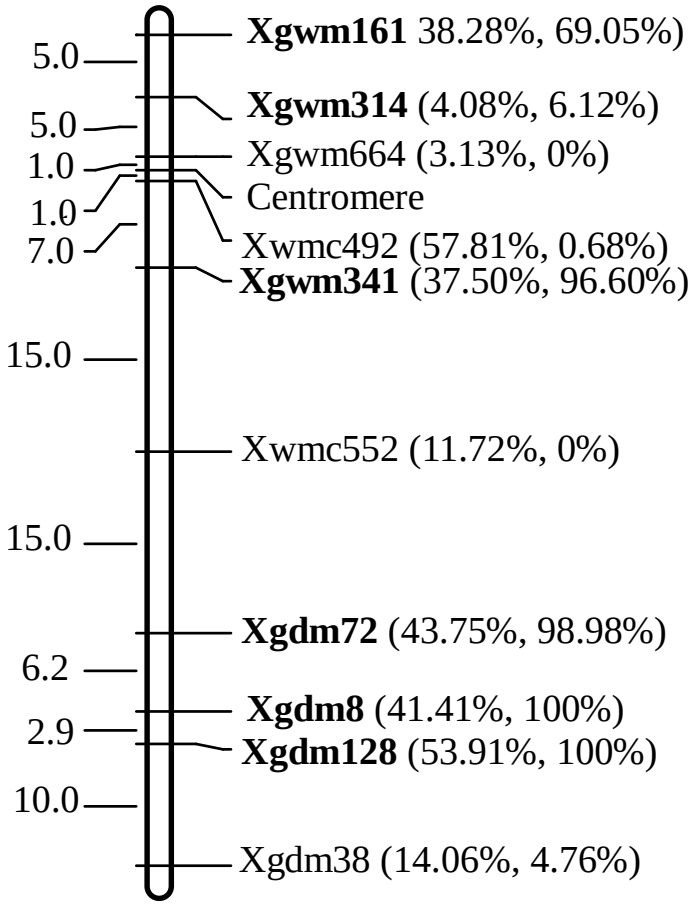

Figure 1. Comparison with distribution frequencies of *A. tauschii* introgression loci on 3D chromosome. The front percent and the back one in the bracket means the distribution frequency of *A. tauschii* introgression loci on certain locus between BC_1F_2 and BC_1F_2-F_7.

population (BC_1F_2 population) with advanced lines (BC_1F_2-F_7 population). The distribution frequencies of 79.41% introgression loci were changed significantly. Of 79.41% changed introgression loci, 22.06% loci significantly increased and 57.35% loci reduced significantly. Zhang et al. (2005) found that the allele of frequencies were deviated from expected segregation ratios in the synthetic backcross-derived lines, were linked to chromosomal regions controlling the traits under selection. In present study, seven selective advantage loci from 3D, 5D and 6D chromosomes of *A. tauschii* were observed, which distribution frequencies were increased from 29.69 to 53.91% in the original BC_1F_2 population to 94.22 to 100% in BC_1F_2-F_7 advanced lines. One chromosomal region covering 9.1 cM in 3D (xgdm72- xgdm8- xgdm128) with selective advantage was also detected. Significant deviations of the expected allele frequencies in selective population can be used to analyze desirable traits linked to genes (Harr et al., 2002; Zhang et al., 2005). Therefore, the seven selective advantage loci and one chromosomal region found in present study could be used to evaluate the desirable genes or traits derived from *A. tauschii* SQ-214.

ACKNOWLEDGEMENTS

This project was funded by National basic research program of China (2011CBB100100), the earmarked fund for Modern Agro-Industry Technology Research System, China (nycytx-03), National Natural Science Foundation of China (30771338, 30700495) and the Science and Technology Department of Sichuan Province. The authors are grateful to Dr. Mujeeb-Kazi A of CIMMYT for kindly providing the *A. tauschii* SQ-214.

REFERENCES

Assefa S, Fehrmann H (2004). Evalution of *Aegilops tauschii* Coss. for resistance to wheat stem rust and inheritance of resistance genes in hexaploid wheat. Genet. Resour. and Crop Evol. 51(6): 663-667.

Bassam BJ, Caetano-Anolles G, Gresshoff PM (1991). Fast and

sensitive silver staining of DNA in polyacrylamide gels. Anal. Biochem., 196: 80-83
Giles RJ, Brown TA (2006). Glu-Dy allele variations in *Aegilops tauschii* and *Triticum aestivum*: implications for the origin of hexaploid wheats. Theor. Appl. Genet. 112: 1563-1572.
Friesen TL, Xu SS, Harris MO (2008). Stem rust, stan pot, *Stagonospora nodorum* bloth,and hessian fly resistance in Langdon durum-*Aegilops tauschii* synthetic hexaploid wheat lines. Crop Sci. 48:1062-1070.
Harr B, Kauer M, Schlotterer C (2002). Hitchhiking mapping: a population based fine-mapping strategy for adaptive mutation in Drosophilamelanogaster. Proc. Natl. Acad. Sci. USA. 99: 12949-12954.
He LQ, Tang RH, Gao GQ (2005). Molecular evidence for gene introgression from wild species to cultivated varieties in peanut. Mol. Plant Breed. 3(6): 815-820.
Liu DC, Zhang LQ, Yan ZH, Lan XJ, Zheng YL (2010). Stripe rust resistance in *Aegilops tauschii* and its genetic analysis. Genet. Resour. Crop Evol. 57: 325-328.
Liu RH, Meng JL (2003). MapDraw: a Microsoft Excel macro for drawing genetic linkage maps based on given genetic linkage data. Hereditas (Beijing). 25: 317–321
Liu SB, Zhou RH, Dong YC, Li P, Jia JZ (2006). Development utilization of introgression lines using a synthietic wheat as donor. Theor. Appl. Genet. 112: 1360-1373.
Lu CM, Yang WY, Zhang WJ, Lu BR (2005). Development of allelic specific PCR markers for the high molecular weight glutenin subunit *Dx^{t}1.5* from *Aegilops tauschii* through sequence characterization. Cereal Sci. 41: 13-18.
Mattew NR, Eric LO, Bikram SG, Michael OP, Yue J (2011). Stem Rust Resistance in *Aegilops tauschii* Germplasm. Crop Sci.51: 2074-2078.
Mujeeb-Kazi A, Rosas V, Roldan S (1996). Conservation of the genetic variation of *Triticum tauschii* (Coss.) Schmalh(*Aegilops squarrosa* auel. Non L.) in synthetic hexaploid wheats (*T. turgidum* L. S. lat.×*T. tauschii*; 2n=6x=42, AABBDD) and its potential utilization for wheat improvement. Genet. Resour. Crop Evol. 43: 129-134.
Naghavi MR, Aghaei MJ, Taleei AR, Omidi M, Mozafari J, Hassani ME (2009).Genetic diversity of the D-genome in *T.aestivum* and *Aegilops* species using SSR markers. Genet. Resour. Crop Evol. 56: 499-506.
Pestsova E, Ganal MW, Röder MS (2000). Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome. 43: 689-697.
Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998). A microsatellite map of wheat. Genetics. 149: 2007-2023.
Somers DJ, Isaac P, Edwards K (2004). A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 109(6): 1105-1114.
Valkoun JJ (2001). Wheat pre-breeding using wild progenitors. Euphytica. 119: 17-23.
Yang WY, Yu Y, Zhang Y, Hu XR, Wang Y, Zhou YC, Lu BR (2003). Inheritance and expression of stripe rust resistance in common wheat (*Triticum aestivum)* transferred from *Aegilops tauschii* and its utilization . Hereditas.139: 49-55
Zhang PZ, Dreisigacker S, Melchinger AE, Reif JC, Mujeeb-Kazi A, Van-Ginkel M, Hoisington D, Warburton ML (2005). Quantifying novel sequence variation and selective advantage in synthetic hexaploid wheats and their backcross-derived lines using SSR markers. Mol. Breed. 15: 1-10.
Zhu MY, Wang YY, Zhu YY, Lu BR (2004). Estimating genetic diversity of rice landraces from Yunnan by SSR assay and It's implication for conservation. Acta Botanica Sinica. 46(12): 1458-1467.
Zhu LC, Smith CM, Fritz A, Boyko E, Voothuluru P, Gill BS (2005). Inheritance and molecular mapping of new greenbug resistance genes in wheat germplasms derived from *Aegilops tauschii*. Theor. Appl. Genet. 111: 831-837.