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Genetic diversity of Triticale genotypes collected from Australia and different area of China

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Introduction

The small grain cereal triticale (*Triticosecale* Wittmack; $2n = 6x = 42$), a man-made wheat-rye hybrid, is considered a promising crop due to its high genetic variation for several traits of agronomic importance. It is widely adapted to abiotic stress conditions such as cold, drought, salinity, and acidic or waterlogged soils (Badea *et al*., 2011). With regard to biomass yield, triticale is able to produce more biomass for a comparable grain yield than other crops (Alheit *et al*., 2011).

The alpine pasture of the Qinghai-Tibet plateau (QTP) is regarded as the most unique alpine pasture ecosystem in the world due to its coverage (257 000 km^2, ~25% of the total area of China) and high elevation (4000 m on average) (Li *et al*., 2012). It is also the feed base for the grassland animal husbandry in China (Zhou *et al*., 2005). Triticale has high forage yield varying from 13.5 to 17.8 t/ha in this area due to its strong cold resistance. Besides, the crude protein content in the forage varies from 12 to 17% (Zhao, 2015). In this paper, genetic diversity of the triticale genotypes was studied using ISSR marker. The results would lay foundation to the triticale breeding in QTP and give useful information on the selection of suitable parents to breed new triticale variety.

Materials and Methods

Thirty triticale genotypes (Table 1) collected from Australia and China were planted on the farm of Gansu Agricultural University on March 8, 2014. All these genotypes had strong cold resistance and well adapted to the climate of QTP. Three grams of leaf of each genotype was collected on April 25, 2014. DNA was extracted from the 30 triticale genotypes using the CTAB method with modifications and quantified using a spectrophotometer and diluted to 25 ng/μl before using.

Inter-simple sequence repeats (ISSR) marker was used to analyze the genetic diversity of the 30 triticale genotypes. PCR amplification reactions were carried out in 20 μl volume, containing 1× buffer, 0.2 mM each dNTP, 0.65 μM primer, 1.9 mM $MgCl_2$, 2 U Taq DNA polymerase, and 50 ng template DNA. PCR amplification was performed as follows: pre degeneration 5 min at 94°C; degeneration 30 s at 94°C, annealing 30 s at 52.3℃, extension 1 min at 72°C, 30 cycles; and extension again 7 min at 72°C.

Table 1: Name and Origin of the 30 triticale genotypes

No.	Genotype	Origin	No.	Genotype	Origin
1	T17	University of Sydney, Australia	10	8809	North-east University, China
2	T6	University of Sydney, Australia	16	HH124	Crop Institute of Chinese Agricultural Academy, China
3	Xinmai No.4	Shihezi University, China	17	HH127	Crop Institute of Chinese Agricultural Academy, China
4	Xinmai NO.5	Shihezi University, China	19	PH389	Crop Institute of Chinese Agricultural Academy, China
5	Bei B-26	Jiusan Institute of Heilongjiang province, China	20	DH796	Crop Institute of Chinese Agricultural Academy, China
9	89D-8	Jiusan Institute of Heilongjiang province, China	21	An 83-25	Crop Institute of Chinese Agricultural Academy, China
12	84B-141	Jiusan Institute of Heilongjiang province, China	22	Zhongla No.1	Crop Institute of Chinese Agricultural Academy, China
13	Beilian No.1	Jiusan Institute of Heilongjiang province, China	23	OH2372	Crop Institute of Chinese Agricultural Academy, China

14	Beilian No.3	Jiusan Institute of Heilongjiang province, China	24	OH1194	Crop Institute of Chinese Agricultural Academy, China
15	Beilian No.5	Jiusan Institute of Heilongjiang province, China	25	OH2276	Crop Institute of Chinese Agricultural Academy, China
18	Beilian No.4	Jiusan Institute of Heilongjiang province, China	26	OH1859	Crop Institute of Chinese Agricultural Academy, China
6	83P-9	Agricultural Institute of Inner Mongolia, China	27	OH1411	Crop Institute of Chinese Agricultural Academy, China
7	81S28	Agricultural Institute of Inner Mongolia, China	28	OH1181	Crop Institute of Chinese Agricultural Academy, China
8	826126	Agricultural Institute of Inner Mongolia, China	29	OH2236	Crop Institute of Chinese Agricultural Academy, China
11	81S37	Agricultural Institute of Inner Mongolia, China	30	OH2473	Crop Institute of Chinese Agricultural Academy, China

Results and Discussion

Twelve primers that could amplify reproducible and clear amplification products were selected in this study. The 12 primers produced 124 bands across 30 genotypes, of which 9 were polymorphic. The number of bands varied from 7 (UBC857) to 15 (UBC815), and size ranged from 200 to 2,200 bp. The average number of bands and polymorphic bands per primer was 10.33 and 8, respectively. The percentage polymorphism ranged from 33.3% (UBC825) to 100% (UBC808), with an average of 77.42% across all the genotypes. These showed that high genetic diversity at the molecular level existed for 30 triticale genotypes.

The ISSR bands were scored for presence or absence among the genotypes and used for the UPGMA cluster analysis. A dendrogram based on UPGMA analysis with ISSR data was produced, with Jaccard's similarity coefficient from 0.66 to 0.91 (Fig. 1).

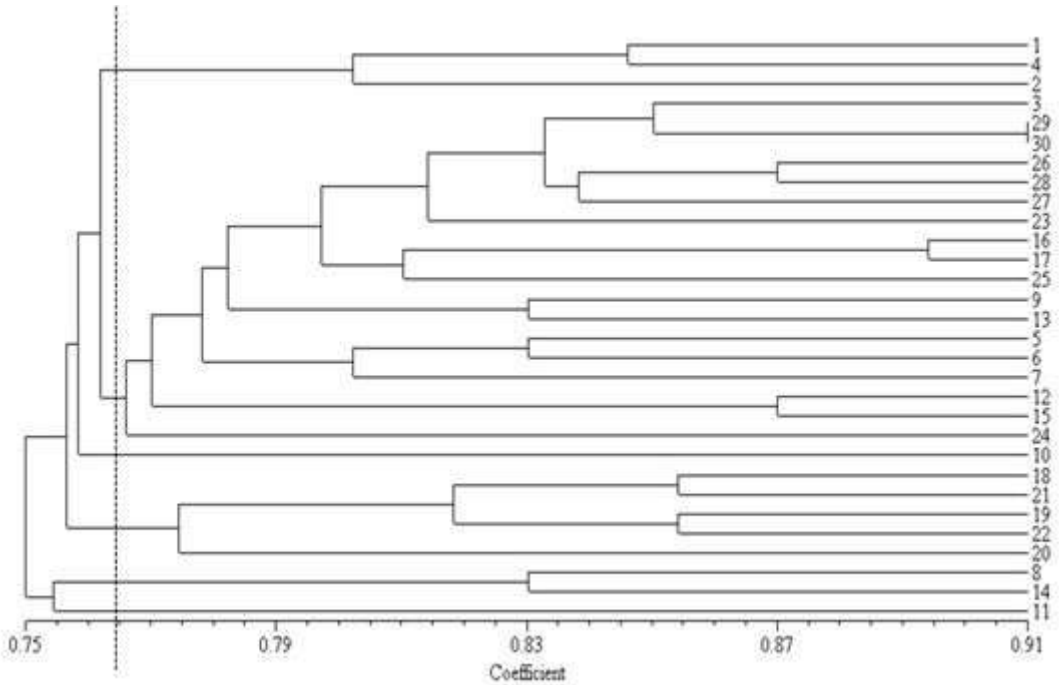

Fig. 1: UPGMA dendrograms of 30 triticale genotypes based on Jaccard's genetic indices using ISSR marker.
The number 1-30 in the above figure means the triticale number in Table 1.

The 30 genotypes clustered into 6 groups. Genotype 8809 (from North-east University, China) was clustered into Group III and 81S37 (from Agricultural Institute of Inner Mongolia, China) was in Group = 6 * ROMAN VI. Group = 5 * ROMAN V included genotype 826126 (from Agricultural Institute of Inner Mongolia, China) and Beilian No.3 (from Jiusan Institute of Heilongjiang province, China). Group I comprised Australian triticale genotypes T17 and T6, and Xinmai No.5 from Shihezi University, China. Group = 4 * ROMAN IV included Beilian No.4 from Jiusan Institute of Heilongjiang province, PH389, DH796, Zhongla No.1 and An 83-25 from Crop Institute of Chinese Agricultural Academy. The others, including 8 from Crop Institute of Chinese Agricultural Academy, 5 from Jiusan Institute of Heilongjiang province and 2 from Agricultural Institute of Inner Mongolia were clustered into group = 2 * ROMAN II.

Among which, close relationship existed between OH2236 and OH2473, HH124 and HH127, OH1859 and OH1181, and 84B-141and Beilian No.5, and the genetic similarity coefficients were 0.91, 0.90, 0.887 and 0.87, respectively. The dendrogram results demonstrated that close relationships existed for the triticale genotypes originated from the same area, such as T17 and T6, OH2236 and OH2473, and HH124 and HH127 *et al*. Maybe they were the crossing offspring of the same parents or one of the parents was the same (Badea *et al*., 2011). But for the genotypes such as Beilian No.3 and No.4, 8809 and 81S28, and Zhongla No.1 and OH1194, they had low genetic similarity although they came from the same regions. These demonstrated that no strict *consistency* existed between the genetic relationship and origin.

Genotypes, such as 81S28, Beilian No.5, and Xinmai No.5, could be used as excellent parents to breed new triticale varieties because these 3 genotypes had high forage yield in QTP (data not shown) and abundant genetic diversity existed for them.

Conclusion

The present study found high genetic diversity at the molecular level for 30 triticale genotypes. The genetic similarity coefficient varied from 0.66 to 0.91 and the average was 0.77. Genotypes 81S28, Beilian No.5, and Xinmai No.5 had high forage yield in QTP and abundant genetic diversity. These 3 genotypes could be used to breed new triticale varieties.

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