Transferring of the biological nitrification inhibition (BNI) character from *Leymus racemosus* to wheat

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ABSTRACT

Biological nitrification inhibition (BNI) is a character that may result in a reduction of emissions of nitrous oxide (N_2O), a green house gas that has more than 300 times the warming power of CO_2, as well as other forms of N which are lost to the environment. The BNI character has not been found in the three major crops; wheat, rice and maize. However, *Leymus racemosus*, alien species of wheat, has shown high BNI capacity. One of *L. racemosus* chromosome addition lines of wheat, Lr#n chromosome addition line, expressed about 80% of BNI character of *L. racemosus*, showing that BNI can be transferred into a wheat background. Two other addition lines showed higher levels of BNI than the parental wheat line. To introduce the BNI character into wheat cultivars, two translocation lines of Lr#n chromosome have been produced.

INTRODUCTION

For wheat production, about 1/3 of the world's nitrogen fertilizer production is applied to wheat crops, but nitrogen use efficiency is only about 33% (Raun and Johnson 1999). Nitrification, the conversion from NH_4^+ to NO_3^- or NO_2^- by soil bacteria is one of the major constrains to seek nitrogen use efficiency (Subbarao et al. 2006b). NO_3^- or NO_2^- are also sources of NO and N_2O gases, the latter one is powerful greenhouse gases with GWP (global warming potential) of >300 times that of CO_2. Biological nitrification inhibition (BNI) is a character through which plants could slow down the conversion ($NH_4^+ \rightarrow NO_3^-$ or NO_2^-) by biological means; therefore, BNI could be genetically exploited to improve agronomic nitrogen-use efficiency and contribute towards the reduction of greenhouse gas emissions from agricultural systems in general and wheat production systems in particular. It has been reported that tropical pastures such as *Brachiaria humidicola* has the BNI character but it has not been found in major crops of wheat, rice, and maize (Subbarao et al. 2007). The *Leymus racemosus*, Volga wildrye, (2n=4x=28; genome NsXm) is an alien species of wheat in Triticeae and grows vigorously in nutrition poor areas of coastal and inland dry areas of Eastern Europe and Central Asia, which would suggest some expectation on nutrition use efficiency.

MATERIALS AND METHODS

Plant materials

Two varieties of bread wheat, Chinese Spring (CS) and Nobeokabouzu, were used for the study. One accession of *Leymus racemosus* (Lam.) Tzvelev was collected along the Black Sea coast (accession number HT15405) and has maintained as clones. The *L. racemosus* chromosome addition or substitution lines were produced in Tottori University, Japan (Kishii et al. 2004) or provided from the Wheat Genetic and Genomic Resources Center (WGGRC), Kansas State University (Qi et al. 1998). Chinese Spring monosomic 3B, 5B, and 7B lines (2n=41) and *ph1b* line have been maintained at the International Maize and Wheat Improvement Center (CIMMYT).

BNI analysis

The detail description of BNI analysis could be found in the previously published article Subbarao et al. 2007. In short, plants were grown in nutrient solution to collect root exudates. The exudate, after concentrating, was applied to bacterial bioassay system of recombinant luminescent *Nitrosomonas europaea*. *N. europaea* is a soil bacteria that oxidizes NH_4^+ to NO_2^-, and the effect of BNI from plants was measured as amount of luminescent emitted from the recombinant bacteria. The inhibitory effect from 0.22 mM allylthiourea (AT), that is one chemical substance to inhibit the growth of *N. europase*, is defined as one AT unit of activity and the inhibitory effect of root exudate is expressed in AT units (Subbarao et. al. 2006a).

Production of Robertosonian (centromeric) translocation lines of Lr#n chromosomes

The addition line, DALr#n, was crossed with CS monosomic 3B and 7B lines. In F1, the plants with 42 chromosomes were selected and then the presence of one Lr#n chromosome and one of corresponding 3B or 7B chromosome were confirmed with C-banding. The translocations between Lr#n and one of the corresponding B genome chromosomes were screened in F2 with Genomic *in situ* hybridization with genomic DNA of *L. racemosus* as probe.

RESULT AND DISCUSSION

Evaluation of BNI

BNI capacity of wheat was from 1-6 AT units g^{-1} root dry weight, while it was 27-31 AT in *L. racemosus* (Table 1), which is comparable to the highest level in the plant (Subbarao et al. 2007). To find out which chromosome was responsible for BNI as well as expression in wheat background, we analysed BNI of *L. racemosus* addition and substitution lines (Table 1). One addition line, DALr#n (disomic addition line of *L. racemosus* Lr#n chromosome), showed about 80% of the capacity of the parental *L. racemosus* and two other addition lines, DALr#I and DALr#J showed about twice the value than the parental wheat line. It is indicating that BNI of *L. racemosus* is a complex trait, but there would be major gene(s) in Lr#n chromosome of *L. racemosus*, which can express in wheat background.

The BNI levels found in the addition/substitution lines of chromosomes DA2Lr#1/DS2Lr#1(2B) or DALr#H/DSLr#H(3A) were different. In both cases, 2Lr#1 and Lr#H chromosome, the substitution lines showed higher BNI than the corresponding addition lines. One possible explanation could be that wheat could have been masking factors/genes for BNI production in the replaced chromosomes.

Production of translocation lines

Since it was reported that Lr#n chromosome had hybridized RFLP markers of group #3 and 7 chromosomes (Kishii et al. 2004), we crossed DALr#n line with monosomic Chinese Spring 3B and 7B chromosome lines to produce Robertsonian (centromeric) translocation. We also separately obtained one naturally occurring substitution line of Lr#n chromosome with 3A chromosome (data not shown), meaning that Lr#n chromosome would be more compensate or have homology to the group 3 chromosome of wheat. Both short and long arm translocation were obtained for 3B and 7B chromosomes (Table 2). More number of short arm translocations was obtained in both 3B and 7B chromosomes, but there were no significant differences in the frequency between 3B and 7B chromosomes. Only the short arm translocations were succeeded to be disomic condition for the both 3B and 7B chromosome translocation. All translocations have been backcrossed with modern varieties two-three times to change wheat background. Meanwhile, CS *ph1b* line was crossed twice to reduce the size of alien segment. The screening of translocation with reduced size is now underway.

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Table 1. BNI released from chromosome addition and substitution lines derived from the inter-specific crosses of the cultivated wheat Chinese Spring with *Leymus racemosus*

Lines*	*L.racemosus* chromosome introduced	Homoeologous group to wheat chromosome	BNI released (AT units g^{-1} root dry weight)
L. racemosus (NsXm gnome, 2n=28)			31.55
Wheat cv. Chinese Spring (ABD genome, 2n=42)			6.39
Chromosome addition line lines (2n=44 or 42+2 telosomes)			
DALr#n	Lr#n	Groups 3 and 7	24.57
DALr#J	Lr#J	Group 7	13.47
DALr#I	Lr#I	Group 5	13.02
MALr#E	Lr#E	Group 4	8.98
DA5Lr#1	5Lr#1	Group 5	6.55
DALr#l	Lr#l	Group 2	6.40
DtA7Lr#1-1	7Lr#1-1	Group 7	6.38
DALr#k	Lr#k	Group 6	5.50
DtA7Lr#1-2	7Lr#1-2	Group 7	4.90
DALr#F	Lr#F	Group 4	4.12
DALr#H	Lr#H	Group 3	3.65
DA2Lr#1	2Lr#1	Group 2	3.16
Chromosome substitution lines (2n=42)			
DS2Lr#1(2B)	2Lr#1	Group 2	12.58
DSLr#H(3A)	Lr#H	Group 3	10.70
		LSD (0.05)	3.93

* MA; monosomic addition, DA; disomic addition, Dt; ditelosomic addition, DS; disomic substitution of *L. racemosus* chromosomes to/with Chinese Spring chromosomes.

Table 2. Robertosonian (centromeric) translocation lines of *L. racemosos* Lr#n chromosome obtained from the crosses between DALr#n and monosomic lines

Monosomic line utilized	Number of plants screened	Number of translocations obtained	
		Short arm	Long arm
CS monosomic 3B	108	6	1
CS monosomic 7B	115	8	1