Employment of rye chromatins in the wheat breeding program

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Abstract

Wheat-rye translocations have provided useful genetic sources in wheat improvement. Our researches are mainly focused on (1) development of new lines that give high yield, pest resistance, favorite end-use quality, and other characteristics related to enhanced crop value and production efficiency via wheat-rye translocations, and (2) elucidation of the significant role of translocated rye chromatins, especially 2RL, in the form of wheat-rye translocations. To achieve above research goals, integrative approaches encompassing molecular genetics, genomics, and conventional breeding methodology were applied. Genetic markers such as ESTs-derived 2RL-specific markers were developed and incorporated into breeding programs to facilitate selection. Genome-wide analysis of transcripts in 2BS.2RL wheat-rye translocation was conducted to elucidate systems responsible for the pest resistance.

Introduction

Wheat–rye translocations have been developed as an important genetic source of disease and pest resistance, and superior performance in the unfavorable environments for crop production. Wheat–rye translocations in the form of 2BS.2RL had an improved agronomic performance related to biotic and/or abiotic stresses such as Hessian fly resistance, powdery mildew, and multiple disease resistance. Detection of additional agronomic traits such as reddish glume and awn color were also reported (Lee et al. 2009).

Our research projects are mainly focused on the development of cultivars through introduction of 2RL that possesses *H21* and agronomically important traits such as improved yield and pest resistance.

To achieve above research goals, integrative technologies encompassing development of genetic markers, genome-wide analysis of transcripts, and employment of molecular genetic tools in conventional breeding methodology were applied.

Materials and Methods

Development of ESTs-derived 2RL-specific markers

- In silico data mining: Contig sequences (GrainGenes) and Unigene databases (NCBI & TIGR)
- Usage of wheat progenitors, 2BS.2RL wheat-rye translocations, and wheat-rye addition lines
- Development of unigene-clusters, cross-species markers, and ESTs-derived 2RL-specific markers

Employment of Wheat Genome Array

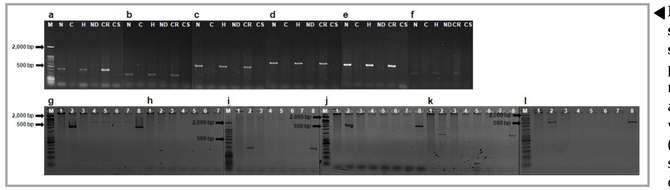
- Affymetrix GeneChip® Wheat Genome Array
- Usage of near-isogenic lines (NILs) (Seo et al. 1997), $BC_3F_{3:4}$ (2BS.2RL: resistant to biotype L of Hessian fly) and 'Coker 797' (non-2RL: susceptible to biotype L of Hessian fly)
- Infestation of biotype L of Hessian fly
- Analysis of differentially expressed transcripts (DETs) and expression level polymorphisms (ELPs)
- Construction of comparative physical map of wheat chromosome arm 2BL, rice chromosome 4, and rye chromosome 2L

Field trials and evaluation of wheat-rye translocations

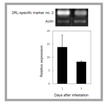
- Fluorescence in situ hybridization analysis
- Application of markers on experimental lines (F_{4-7}) and US breeding materials (UGA-Griffin Campus)

Molecular Genetic Analysis

: Development and functional assessment of ESTs-derived 2RL-specific markers



◀ **Fig. 1 Confirmation of six ESTs-derived 2RL-specific markers.** PCR products of 2RL-specific markers using 2BS.2RLs and non-2RLs (**a-f**), and wheat-rye addition lines (**g-l**). Each primer pairs successfully produced clear unique amplicon in the lines with 2RL and not in the lines without 2RL.



◀ **Fig. 2 Functional assessment of ESTs-derived 2RL-specific marker no. 2 (NSFT03P2_Contig4445) in near-isogenic line (NIL) possessing *H21*.** RT-PCR analysis was performed in Hessian fly infested NIL (2BS.2RL). real-time RT-PCR was performed in Hessian fly infested and non-infested NIL (2BS.2RL). Expression of 2RL-specific marker no. 2 in Hessian fly non-infested samples was defined as 1. The early burst of gene expression at the initiation of larvae attack in the 2BS.2RL wheat-rye translocation was detected.

Genomic Analysis

: Wheat genome array

Fig. 3 Graphical view of expression level polymorphisms (ELPs) and differentially expressed transcripts (DETs) in resistant and susceptible near-isogenic lines (NILs) under Hessian fly infestation. 33 ELP groups (A to AG) were arranged in clockwise rotation. Re: DETs in resistant NIL, Su: DETs in susceptible NIL. Identical transcripts among ELP groups and transcripts observed in resistant or susceptible NIL were linked by gray lines. ELPs and/or transcripts matching to rice syntenous group 4 or 7 were linked by blue or black line, respectively.

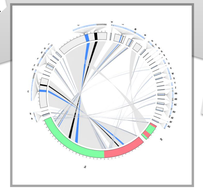
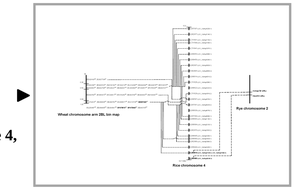
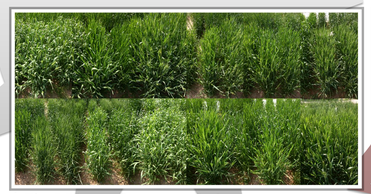


Fig. 4 Gene sets-synteny-based comparative physical map of the wheat chromosome arm 2BL, rice chromosome 4, and rye chromosome 2.

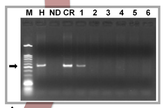


Application to Conventional Breeding Methodology

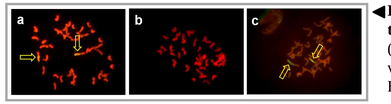
: Field trials and evaluation of wheat-rye translocations



▲ **Fig. 5 Plot performance of wheat-rye translocations at heading date in Deockso, Korea.**



▲ **Fig. 6 Marker application to verify the presence of 2RL in US breeding materials.** A ESTs-derived 2RL-specific marker was amplified in *H* ('Hamlet': 2BS.2RL), *CR* ('Chaupon' rye), and lane 1.



◀ **Fig. 7 FISH of 2BS.2RL wheat-rye translocations. a** FISH of NIL (2BS.2RL). **b** FISH of normal hexaploid wheat 'Coker 797' (normal 2BL). **c** FISH of 'Hamlet' (2BS.2RL).

Results

ESTs-derived 2RL-specific markers were developed (Fig. 1). The significant transcriptional increases of one of the 2RL-specific markers were functionally assessed under Hessian fly infestation (Fig. 2).

Wheat Genome Array revealed Hessian fly infestation-specific transcripts as well as basal defense-mediated transcripts (Fig. 3).

The application of Wheat Genome Array for high variance probe set analysis was conducted on NILs. Comparative physical map of wheat chromosome arm 2BL with homology to rice chromosome 4 and rye chromosome 2 was constructed (Fig. 4).

Discussion and Conclusion

Our research utilizes appropriate technologies encompassing molecular genetics, genomics, and conventional breeding methodology that contribute to the development of high quality and stress tolerance wheat cultivars. Integration of those information would enhance the usefulness of rye chromatins for wheat improvement.

References

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Seo YW, Johnson JW, Jarret RL (1997) A molecular marker associated with the H21 Hessian fly resistance gene in wheat. Mol Breed 3:177-181

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