Molecular diversity and association mapping in a collection of synthetic hexaploid wheat

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INTRODUCTION

Overcoming abiotic and biotic stresses, including increased incidence of diseases, are some of the major challenges facing plant breeders addressing sustainable wheat production in the 21st century. The availability of effective disease resistance genes has been further reduced over time as pathogens, particularly rusts, have evolved to acquire virulence on previously effective genes. The effective control of diseases of cereals, in particular wheat, is critical to maintaining stability in global food supplies. In many cases the availability of genetic resistance is limited because of the lack of genetic diversity within the cultivated species. Synthetic hybrid wheats (SHW), created by artificially crossing durum wheat (*Triticum turgidum*; 2n=4x=28 AABB), donor of the bread wheat A and B genomes, with accessions of *Aegilops tauschii* (2n=2x=14 DD), donor of the D genome, mostly developed over the last twenty years at the International Maize and Wheat Improvement Center (CIMMYT), have become exciting new sources of resistance to biotic stresses (Ogbonnaya et al 2008). One such disease is cereal cyst nematode (CCN, *Heterodera avenae*) which can reduce yields of wheat crops by much as 50%, particularly in infested soils where cereal crops are produced in consecutive years or when cereals are planted following broadleaf crops invaded by grass weeds. Genetic resistance is the preferred means of disease control because it avoids the use of pesticides, is cost effective and is a low technological management option. Resistance is particularly important to farmers in developing countries where food production is less advanced and much of the world's wheat is produced.

Numerous marker technologies have been developed over the past two and half decades. Recently, diversity arrays technology (DArT) (Jaccoud et al. 2001) has been used to study genetic diversity and genetic mapping in a range of crops including wheat (Hearnden et al. 2007). DArT simultaneously types several thousand loci in a single assay. DArT generates whole-genome fingerprints by scoring the presence versus absence of DNA fragments in genomic representations generated from samples of genomic DNA.

The objectives of this study were to analyse the molecular diversity in a collection of SHWs, and explore the use of DArT markers for association mapping of cereal cyst nematode resistance. This would provide a guide to the exploitation of this set of SHWs for other agronomically important traits for which they have been evaluated.

MATERIALS AND METHODS

Plant materials

Seventy six wheat lines comprised of 61 SHWs, 8 durum tetraploid wheats, five elite Australian bread wheat cultivars and two diploid *Ae. tauschii* accessions were analyzed. The SHWs were produced from 52 and 27 *Ae. tauschii* and elite durum parents respectively, by scientists at CIMMYT, Mexico and the Victorian Department of Primary Industries, Australia. All the SHWs, resistant and susceptible check varieties were evaluated for CCN resistance.

Disease assessment for CCN resistance

The CCN inoculum preparation and subsequent method of evaluation was described by Ogbonnaya *et al.* (2001).

DNA isolation and Marker characterization

For DNA extraction, the 94 lines were planted in the green-house. Between 2-3 weeks after planting, young leaves were harvested from five plants per line, frozen in liquid nitrogen and stored at -80°C prior to DNA extraction. Genomic DNA was extracted according to Ogbonnaya et al. (2001). Ten microliters of 100 ng/µl DNA from each sample (94 lines with controls duplicated at the beginning and middle of the 96-well tray) was sent to Triticarte Pty Ltd (http://www.triticarte.com.au;Yarralumla, Australia). DArT® markers were scored on the 94 lines by Triticarte Pty Ltd, which probed genomic DNA from the individual 94 lines against the wheat DArT® array. A total of 493 DArT® informative markers were scored which spanned 21 wheat chromosomes with marker numbers ranging from 5 on chromosome 6D to 61 markers on chromosome 3B.

Allele diversity

Gene diversity and polymorphic information content (PIC) values for each of the DArT loci were calculated using PowerMarker v3.07 (Liu and Muse 2005).

Population structure and marker-trait analysis

Possible population structure was investigated by using the Bayesian and neighbour clustering methods. The former was implemented in the software program STRUCTURE 2.1 (Pritchard et al. 2000) using 493 mapped DArT markers and one gene-based STS marker. The basis of the Bayesian clustering method is the allocation of individual genotypes into groups in such a way that Hardy-Weinberg equilibrium and linkage equilibrium are valid within clusters and absent between clusters. The optimum number of genetic populations (K value), based on Lnpd values, was selected after twenty independent runs of a burn-in rate of 10,000 cycles, adopting an admixture model. Two genetic structure tests were successively performed for all genotypes (SHWs, commercial bread wheat cultivars, *Triticum turgidum* cv. durum and *Aegilops tauschii)* and for 59 SHWs only (those SHWs with phenotypic data). We used a general linear model (Yu et al. 2006) to test for association between marker data and CCN resistance using the software TASSEL 2 (http://www.maizegenetics.net/tassel) to minimize spurious correlations and the elevated false-positive rate.

RESULTS AND DISCUSSION

1) Genetic variation for CCN resistance

Considerable genetic variation exists in SHWs for CCN resistance against the Australian CCN pathotypes, *Ha13*. Of the SHWs evaluated, 20 exhibited complete resistance, 10 were moderately resistant while the remaining were susceptible. Out of the designated nine resistance genes for CCN, seven originated from wild species including two – *Cre3* and *Cre4* from *Ae. tauschii*. Others were from *Ae. ventricose*, *Ae. variabilis*, *T. monococcum*, and *Secale cereale*. The additional sources of resistance identified in SHWs could be explored as possible sources of novel genes to increase genetic diversity for resistance to CCN in bread wheat.

2) Allelic diversity

The PIC value ranged from 0.0512 to 0.3750 with a mean of 0.2854. The low PIC values for DArT markers in wheat were lower than that reported for cassava with an average PIC value of 0.42 (Xia et al. 2005). Possible reasons for the low PIC values in the current study include the inherent nature of DArT and the limited tetraploid parents used in producing the SHWs.

Principal Coordinate Analysis (PCoA) shows complete distinction between SHWs, durums, *Ae. tauschii* and commercial bread wheat cultivars (Figure 1), consistent with neighbour joining dendrogram (Figure 2). These show the separation of the SHWs, durums, *Ae. tauschii* and commercial bread wheat cultivars in diversity space on a whole genome basis and are entirely consistent with the genetic history of wheat.

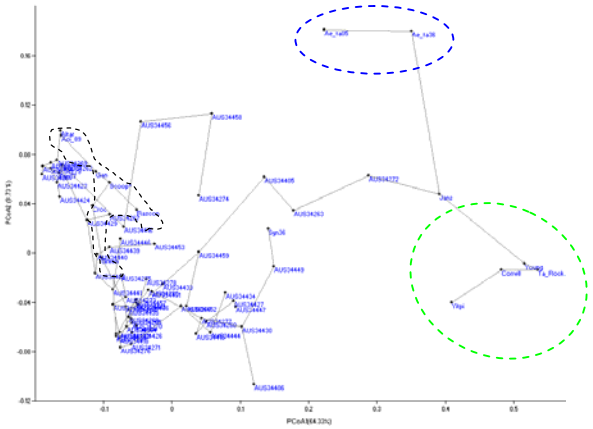

Figure 1. PCoA associated with minimum spanning tree for SHWs, Australian commercial bread wheats, *T. turgidum* cv durum and *Ae. tauschii*.

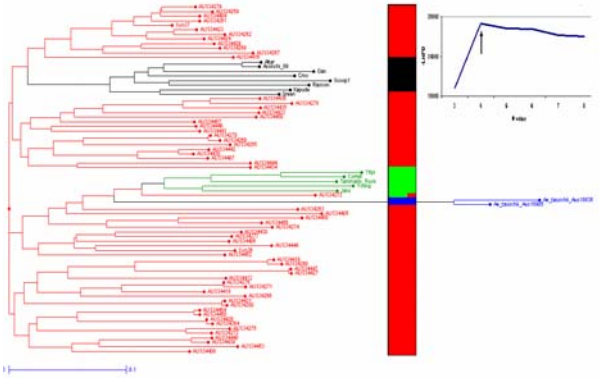

Figure 2. Neighbour joining tree for a set of 76 wheats comprising SHWs (red), *T. turgidum* cv. durums (black), *Ae. tauschii* (blue) and commercial Australian bread wheat cultivars (green).

Table 1. General Linear Model of DArT markers linked to CCN resistance in SHWs.

Locus	Chromosome	#perm_ Marker	p-value	R^{2b}
wPt-1140	2B	1000	0.01	8
Cre3sp	2D	1000	0.0001	23
wPt-9848	2D	1000	0.002	14
wPt-9258	3D	1000	0.005	14
wPt-7265	3D	1000	0.004	13
wPt-2782	4A	1000	0.002	18
wPt-5434	4A	1000	0.01	8
wPt-5256	6B	1000	0.0001	15
wPt-8814	6B	1000	0.003	14
wPt-1241	6B	1000	0.01	11

[b]The amount of genetic variance which would be explained by a DArT/STS marker at this locus, as a percentage.

3) Association mapping for CCN resistance

We detected significant association between CCN resistance with DArT markers on chromosomes 2B, 2D, 3D, 4A and 6B with explained phenotypic variation ranging from 8 to 18%. Previous studies have identified and mapped CCN resistance genes *Cre1* in *Triticum aestivum* on 2B (Williams et al. 1994), *Cre2* in *Ae. ventricosa* on 5Nv) (Delibes et al. 1993, Ogbonnaya et al 2001), *Cre3* and *Cre4* from *Ae. tauschii* on 2D, (Eastwood et al.1994), *Cre5* from *Ae. ventricosa* on 2A, (Jahier et al. 2001), *Cre6* (*Ae. ventricosa* 5Nv) (Ogbonnaya et al. 2001), *Cre7* in *Aegilops truincialis* (Romero et al. 1998) and *Cre8* in *T. aestivum* on 6B (Williams et al. 2003). Additional to the DArT markers associated with CCN resistance in previously reported chromosome regions linked to CCN resistance, we uncovered other potentially novel loci on chromosomes 3D and 4A significantly associated with CCN resistance in SHWs. The gene-based STS marker, *Cre3sp* on chromosome 2DL showed the most significant association explaining 23% of the phenotypic variation in CCN resistance amongst the SHWs.

4) Conclusion

We found significant association between CCN resistance and DArT markers in SHWs. This study provides evidence that association mapping can be utilized to detect loci associated with CCN resistance in SHWs and can be used to uncover novel genetic loci linked to traits of agronomic importance in SHWs. This new diversity could be immediately used for wheat germplasm enhancement.

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