

Genetic diversity and population structure analyses among Indian bread wheat cultivars

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ABSTRACT

A research project on association mapping in wheat is under way in our laboratory. As a first step towards meeting this goal, we carried out genetic diversity and structure analyses in a collection of 134 Indian wheat cultivars that were released over a period of ~100 years (1910 to 2006); 31 developed during pre-green revolution and 103 developed during post-green revolution periods. A set of 42 SSRs (one from each arm of 21 individual chromosomes) with a total of 258 alleles (mean 6.2; range 2-13/SSRs) was used. As many as 65 (25.1%) of the 258 alleles were rare alleles occurring at a frequency of <5%. The average number of alleles/locus (4.99 vs. 4.77) and the estimates of genetic diversity (0.65 vs. 0.63) in cultivars from the pre-and post-green revolution periods did not differ significantly indicating that green revolution did not lead to any loss of genetic diversity. The proportion of the variance among cultivars within groups accounted for 94.4% (as revealed by AMOVA). As many as 10 sub-populations were identified through structure analysis, which included 2 sub-populations with cultivars mainly from pre-green revolution period and 8 sub-populations with cultivars mainly from post-green revolution period.

INTRODUCTION

Genetic improvement of crop plants including wheat relies heavily on the availability of diverse and superior genes/alleles. Wheat germplasm representing wide spectrum of genetic variability for different traits is also useful for identification of marker-trait associations through association mapping. It has, however, been argued that modern plant breeding has led to narrowing of genetic diversity in crop cultivars leading to their genetic homogeneity and possible vulnerability to biotic and abiotic stresses¹⁻⁴. Thus, it is important to understand the genetic structure and level of diversity in the available wheat cultivars, before initiating meaningful study on marker-trait associations.

In the recent past, genetic diversity in bread wheat germplasm from various sources has been studied using molecular markers. Study of wheat cultivars from U.K. and France and wheat collected from Europe and Asia over a period of time suggested more qualitative than quantitative changes in genetic diversity^{3,5,6}. Similar studies involving CIMMYT-based wheat cultivars and spring wheat from Nordic region belonging to the later

part of the 20th century suggested an increase in genetic diversity^{7,8}. The only report, where reduction in allelic richness was reported involved Canadian hard red spring wheat germplasm released during the past ~150 years^{4,9}. However, information on the level of genetic diversity and population genetic structure is not available for elite Indian bread wheat cultivars. Therefore, we carried out the present study to find out the loss, if any, in genetic diversity following the green revolution and to understand the population structure, before studying marker-trait associations.

MATERIAL AND METHODS

Plant material: A set of 134 elite Indian bread wheat cultivars developed from 1910 to 2006 was used in the present study. Thirty one (31) cultivars were developed before 1965 (the pre-green revolution period), and the remaining 103 cultivars were developed after 1965 (the post-green revolution period).

DNA extraction and SSR analysis: Genomic DNA of each cultivar was extracted using a modified CTAB method¹⁰. Forty-two (42) SSR markers, one SSR from each arm of 21 individual chromosomes, were selected from the wheat microsatellite consensus map¹¹. PCR analysis for SSRs was carried out in Eppendorf Master Cycler following Roder et al.¹² and the amplified products were resolved on MEGA-GEL High Throughput Vertical Unit model C-DASG-400-50¹³.

Genetic analysis: Polymorphic information content (PIC)/SSR was estimated as given elsewhere¹⁴. The number of alleles/locus and the gene diversity (*He*) were calculated using POPGENE version 1.31 (University of Alberta, Edmonton, Alberta, Canada)¹⁵. For precise comparison of number of alleles/locus and *He* for 31 cultivars of the pre-green revolution with those from the post-green revolution, the average number of alleles/locus and *He* for the latter were obtained by drawing 1000 random samples (sample size = 31 cultivars) from 103 cultivars of the post-green revolution period using R software package (<http://cran.r-project.org/>). The relative loss of genetic diversity in pre-green revolution vs. post-green revolution cultivars was estimated following Huang et al.¹⁶. The significance of difference between the average number of alleles in the cultivars belonging to the pre- and post-green revolution periods was tested with paired t-test using the software package SPSS for windows (SPSS, Chicago, IL).

Analysis of molecular variance: Analysis of molecular variance (AMOVA) was carried out using ARLEQUIN version 2.0¹⁷.

Structure analysis: Model-based cluster analysis was performed using the software STRUCTURE version 2.0¹⁸. The number of presumed populations (K) was set from 1 to 15, and each was repeated three to five times. For each run, burn-in and iterations were set to 100,000

and 200,000, respectively, and a model without admixture and correlated allele frequencies was used. When alpha was constant, the run with maximum likelihood was used to assign individual genotypes into groups.

RESULTS AND DISCUSSION

A summary of the results of SSR analysis conducted using 42 SSRs with 134 cultivars is presented in Table 1. The 134 elite Indian bread wheat cultivars carried a total of 258 alleles. The number of alleles/ locus ranged from 2 to 13 with an average of 6.2 alleles/locus. Maximum number of alleles (98) was recorded for sub-genome A and minimum for sub-genome D. The average values of polymorphic information content (PIC)/locus for each sub-genome was in agreement with the number of alleles/locus for the individual sub-genomes. Further, for the whole set of 134 cultivars, significant correlation ($r = 0.77$, $P < 0.001$) between number of alleles/locus and the PIC/locus was in agreement with earlier studies^{3,19,20}. The over all average PIC/locus for the three sub-genomes taken together was 0.674. This is in agreement with the average PIC/locus (0.68) for a set of 69 accessions of bread wheat using 52 SSR markers²⁰. Together these results indicated considerable genetic diversity in Indian bread wheat cultivars developed over the past century.

Table 1. A summary of the results of SSR analysis of 134 elite Indian bread wheat cultivars.

Item	A-genome	B-genome	D-genome	Total
SSRs tried	14	14	14	42
Alleles detected	98	85	75	258
Rare alleles (<5%)	25	20	20	65
Average alleles/locus	7	6.07	5.35	-
Average PIC/SSR	0.761	0.635	0.57	-
PIC range	0.626 to 0.861	0.198 to 0.856	0.138 to 0.858	-

Table 2. Comparison of genetic variation between elite Indian bread wheat cultivars developed during pre- and post-green revolution periods.

Item	Pre-green revolution cultivars	Post-green revolution cultivars	Relative loss of diversity
Total sample size	31	103 (31)*	-
Total number of alleles	203	251 (210)*	-
Allele range	2-11	2-13 (2-13)*	-
Unique alleles	7	55	-
Shared alleles	196	196	-
Average no. of alleles/locus	4.77	5.98 (4.99)*	0.032
Gene diversity (<i>He</i>)	0.63	0.65	0.030

* Results from the resampling method are shown in parenthesis

A comparison of genetic variation for SSRs among 31 cultivars from pre-green revolution period with that among 103 cultivars from post-green revolution period suggested higher genetic diversity among cultivars from the post-green revolution period. When adjustment for

sample size was made, no difference in the level of genetic diversity was noticed between the two groups of cultivars (Table 2). These results together with very low values for both the relative loss of average number of alleles/locus and gene diversity (*He*) also suggested that

green revolution did not lead to loss of genetic diversity (Table 2). This is in agreement with the earlier studies suggesting absence of any loss in genetic diversity in bread wheat following modern plant breeding practices^{3,5,16}. Further, the AMOVA indicated that proportion of the variation among the cultivars developed during the pre- and post-green revolution periods accounted for most (94.4%) of the molecular variance while between the groups variation accounted for only 5.6% variance. The higher proportion of genetic variation within groups may be due to the selection for adaptation to local agronomic conditions while breeding bread wheat cultivars^{18,21}.

Model-based cluster analysis grouped 134 Indian wheat cultivars into 10 genetically distinct sub-populations. Twenty-two (22) of the 31 pre-green revolution cultivars were grouped into two sub-populations while rest of the 9 cultivars were admixed with the 103 post-green revolution cultivars that were grouped into 8 sub-populations. Thus, the population structure need to be taken into account while working out the marker-trait association through association mapping to avoid false positives.

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