

Molecular evaluation of Afghan wheat landraces

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Abstract

The wheat landraces collected by Dr Hitoshi Kihara *et al.* from Afghanistan, the place of secondary origin of wheat, are an untapped genetic resource for mining novel alleles. In this study, approximately 400 landraces were collected from seven agroecological zones and characterized using diversity array technology and single-nucleotide polymorphism markers, as well as diagnostic molecular markers at important loci controlling vernalization (*Vrn*), photoperiod response (*Ppd*), grain colour (*R*), leaf rust (*Lr*), yellow rust (*Yr*), stem rust (*Sr*) and Fusarium head blight (*Fhb*). A genome-wide marker array revealed a large amount of genetic diversity among the landraces, 53% of which were winter types, 43% were either spring types or facultative and 4% were either unknown or had *Vrn-A1c* – a rare spring allele that needs to be confirmed with additional genotyping and phenotyping. At *Ppd*, 97% of the lines carried a photosensitive allele. In the case of grain colour, classification based on dominant or recessive allelic combinations revealed that approximately 39% of the population is characterized by white grain. Four gene-specific markers that were targeted to identify loci for rust and *Fhb* resistance enabled us to identify 17 unique landraces with known resistance genes.

Keywords: Afghan wheat landraces; gene-specific markers; genetic diversity

Introduction

Wheat is the most important crop in Afghanistan and accounts for 77% of the total cereal production in this country (World Bank Report, 2012). However, wheat production has been unstable due to various abiotic and biotic stresses such as drought, yellow rust and Sunn pest (*Eurygaster integriceps*). Although previous studies have reported the importance of landraces for developing crops with drought tolerance (Reynolds *et al.*, 2007), disease resistance (Bonman *et al.*, 2007) and high-quality

traits (Black *et al.*, 2000), caution is necessary to avoid deleterious linkage drag when using landraces in breeding programmes. Therefore, searching wheat landraces to identify novel genes for critical traits is important for future breeding programmes, particularly those of rain-fed wheat improvement in Afghanistan and elsewhere.

The scientific expeditions initiated by the late Dr Hitoshi Kihara in 1955, and followed by those of other Japanese researchers in 1965 and 1978, collected Afghan wheat landraces that are now preserved at the Kihara Institute for Biological Research, Japan. Recent advancements in molecular markers with high-throughput systems show great potential for genome-wide characterization of many crops and plants (Tuberosa *et al.*, 2011). In particular, diversity array technology (DArT) and

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single-nucleotide polymorphism (SNP) markers are gaining importance in germplasm studies following reductions in the cost of genotyping. Irrespective of wheat genome size, many diagnostic markers have been developed for vernalization (*Vrn*), photoperiod response (*Ppd*), grain colour (*R*), leaf rust (*Lr*), yellow rust (*Yr*), stem rust (*Sr*) and Fusarium head blight (*Fhb*) (MAS wheat, <http://maswheat.ucdavis.edu/>; Himi *et al.*, 2011; Liu *et al.*, 2008), which aid in the characterization of any new wheat germplasm. Therefore, the aims of this study were to characterize Afghan wheat landraces using SNP and DArT markers and to identify allelic variation at the *Vrn*, *Ppd*, *R*, *Lr*, *Yr*, *Sr* and *Fhb* loci to find novel germplasm for Afghan wheat breeding programmes.

in this study (Fig. 1). Control samples for each gene were included for diagnostic marker screening. We extracted genomic DNA and used a Genotyping by Sequencing (GBS) 1.0 V array (www.triticarte.com.au) with approximately 50,000 probes. Diversity analysis was carried out using Phylip program. Diagnostic markers for *Vrn*, *Ppd*, *R*, *Lr*, *Yr*, *Sr* and *Fhb* were used after standardizing PCR conditions (Table 1). The growth habits of landraces were classified as winter, spring and/or facultative based on the allelic combination of all the three *Vrn* homoeologous loci. In the case of *Ppd*, lines were categorized as either photoperiod sensitive or insensitive. For rust and *Fhb1* resistance, 'susceptible' landraces were identified based on reported information and the unamplified one reported as an unknown or null allele.

Materials and methods

Afghan wheat landraces numbering 446 (hereafter called 'KAWLR', Kihara Afghan wheat landraces) were used

Results and discussion

Molecular characterization is vital for any germplasm study to reveal the genomic constitution or diversity or

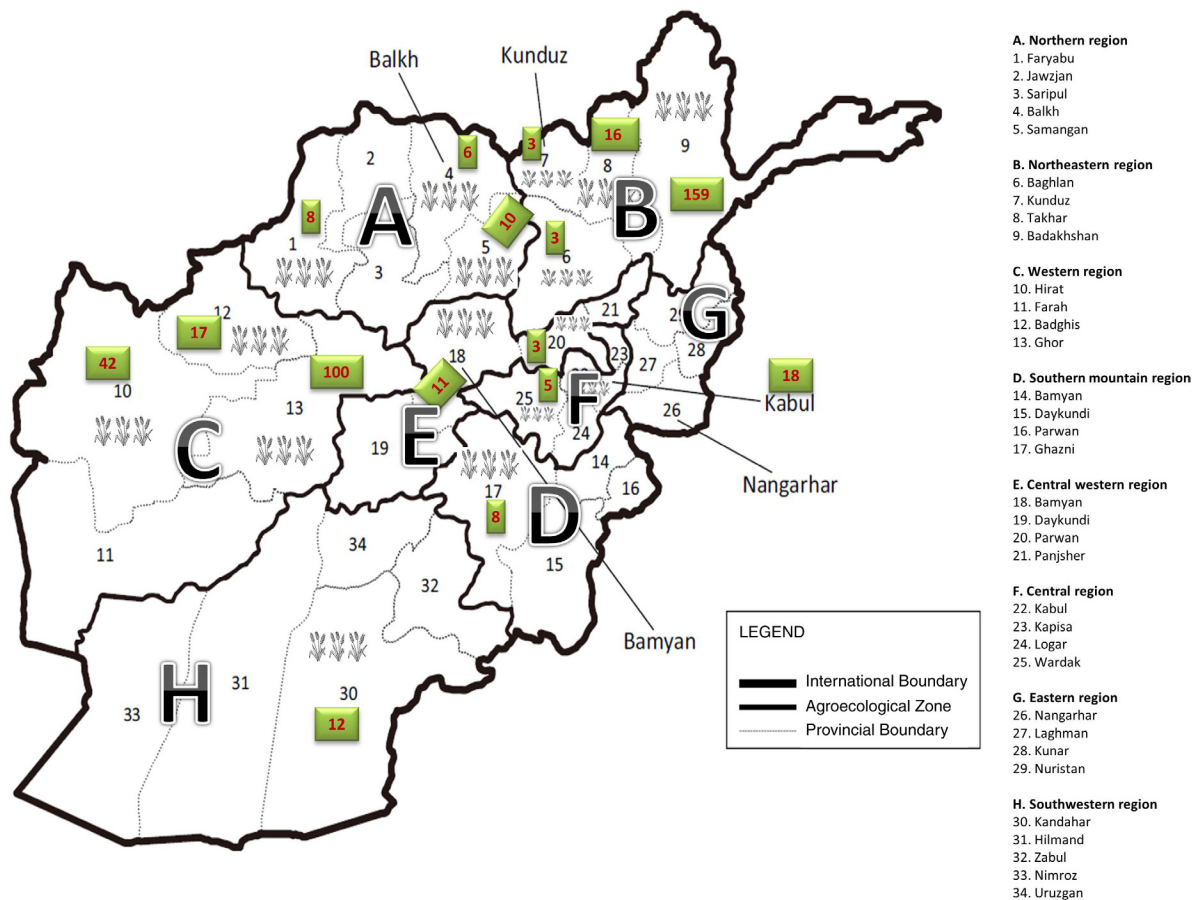


Fig. 1. Afghan wheat landrace collection. Number of lines collected from each province is given in embedded boxes. The whole country is divided into eight agroecological zones (A–H) (FAO Report, 2003) and the provinces under each zone are listed.

Table 1. Diagnostic molecular markers for vernalization (*Vrn*), photoperiod response (*Ppd*), grain colour (*R*), leaf rust (*Lr*), yellow rust (*Yr*), stem rust (*St*) and Fusarium head blight (*Fhb*) genes

Genes	Marker name	Chromosome location	Primer sequences	
			Forward	Reverse
<i>Vrn-A1</i>	VA1-F2 and VA1-R3	5AL	TCAGATTCTAGACTGAGATGTTCAA	GATGTGGCTCACCATCCACG
	VRN1AF and VRN1-INT1R		GAAAGGAAAATCTGCTCG	GCAGGAAATCGAAATCGAAG
	Intr1/A/F2 and Intr1/A/R3		AGCCTCCACGGTTTGAAGTAA	AAGTAAGACAACACGAAATGTGAGA
<i>Vrn-B1</i>	Intr1/C/F and Intr1/AB/R	5BL	GCACTCCTAACCCACTAACC	TCATCCATCATCAAGGCAAA
	(Spring) Intr1/B/F and Intr1/B/R3		CAAGTGGAACGGTTAGGACA	CTCATGCCAAAATTTGAAGATGA
<i>Vrn-D1</i>	(Winter) Intr1/B/F and Intr1/B/F	5DL	CAAGTGGAACGGTTAGGACA	CAAATGAAAAGGAATGAGAGCA
	(Spring) Intr1/D/F and Intr1/D/R3		GTTGCTGCCTCATCAAATCC	GGTCACTGGTGGTCTGTGC
<i>Ppd-D1</i>	(Winter) Intr1/D/F and Intr1/D/R4	2DS	GTTGCTGCCTCATCAAATCC	AAATGAAAAGGAACGAGAGCC
	(Sensitive) 2D Ins F1 and 2D Ins R1		ACGCCTCCCACTACACTG	GTTGGTTCAAACAGAGAGC
<i>RA1: Tamyb10-A1</i>	(Insensitive) 2D Ins F1 and 2D Ins R2	3AL	ACGCCTCCCACTACACTG	CACTGGTGTAGCTGAGATT
	Tamyb10-LP6 and Tamyb10-RP6		ATATGTGGATGGCCTTGGAT	CTACCAGCTCGTTGGGAAG
<i>RA2: Tamyb10-A1</i>	Tamyb10-LP7 and Tamyb10-RP4		TTTCAATCGAGTGGGCATAA	CCTGACGATGAGCTCCTCTT
<i>RA3: Tamyb10-A1</i>	Tamyb10-LP8 and Tamyb10-RP7	3BL	TCCCTAGATGGGACACAGAGA	TGTTATCACATGTGATCCTGA
	Tamyb10-LP2 and Tamyb10-RP1	3DL	AGCAAGAGGAACCTGCAGTC	GATGCCCTCCAGATCAAGGT
<i>RD: Tamyb10-D</i>	Tamyb10-LP9 and Tamyb10-RP3	7DS	TAGGCCAACACCTTCTAAACG	AGGCACACAGCTTATTGG
	csLV34	1BL	GTTGGTTAAGACTGGTGTATGG	TGCTTGTATTGCTGAATAGT
<i>Lr34/Yr18</i>	Xbarc80	6DS	GCGAATTAGCATCTGCATCTGTTGAG	CGGTCAACCAACTACTGCACAAC
<i>Lr46/Yr29</i>	FSD_RSA	3BS	GTTTATCTTTTATTTC	CTCCTCCCCCCCA
<i>SrCad</i>	UMN10		CGTGGTCCACGCTCTCTTA	TGAAGTTATGCCACCAGCATA
<i>Fhb1</i>				

to accelerate the process of further improvement by complementation with impeccable phenotyping. In particular, recent advancement in array-based genotyping is quickening this characterization process with more accuracy (Kilian and Graner, 2012). The GBS array identified 39,855 polymorphic markers in this germplasm collection. The diversity analysis of KAWLR revealed a complex diversity pattern with large diversity within the groups (see online supplementary Fig. S1). Among the 20 clusters, five were prevalent. The major clusters were well aligned with collection sites. Nei's genetic distance analysis indicated that the closest groups were Herat and Ghor, followed by Badakhshan and Takhar with genetic distances of 0.027 and 0.035, respectively. Takhar, however, exhibited the largest genetic distances with Kandahar and Badghis, with values of 0.267 and 0.199, respectively. This result differs from those of previous work on this germplasm reporting a much lower diversity (Terasawa *et al.*, 2009).

The analysis of *Vrn* genes (*Vrn-A1*, *Vrn-B1* and *Vrn-D1*) in a total of 376 wheat landraces revealed a slightly higher frequency of the winter type (55%) than of the spring type. The classification of landraces based on agroecological zones revealed that landraces from the north-eastern and north-western regions possessed winter-type alleles when compared with those from other zones. Winter-type wheat is generally more tolerant to frost than spring-type wheat (Fujita *et al.*, 1992) and is thus better adapted to cold areas with average winter temperatures ranging from -5 to 5°C . In Afghanistan, unlike spring-type wheat, most winter-type wheat is grown under rain-fed conditions. Therefore, the untapped landraces of Afghanistan can be effectively used in winter wheat breeding programmes. Landraces with a spring-type *Vrn* allele (*Vrn-A*, *Vrn-B* or *Vrn-D*) were identified based on the presence of either one or a combination of *Vrn* homoeologous alleles on chromosome 5. The *Vrn-D1* allele was present in 111 landraces followed by *Vrn-B1* (70) and *Vrn-A1* (16) (see online supplementary Table S1). Unexpectedly, a combination of spring alleles was not found to classify the lines with the exception of a few having the combination of *Vrn-A1*, *-B1*, *Vrn-A1*, *-D1* or *Vrn-B1*, *-D1*. The lower frequency of *Vrn-A1* alleles and their combinations suggests that even landraces classified as the spring type need medium-to-mild vernalization. In particular, the spring wheat cultivars carrying the *Vrn-D1* allele for normal flowering may have a higher grain yield in a Mediterranean environment in Turkey (Andeden *et al.*, 2011). The majority (97%) of the landraces are photoperiod sensitive and distributed throughout the country without much dependence on agroecological zones. A similar distribution has been reported in Chinese landraces (Zhang *et al.*, 2010). The combination of two growth habit traits

(i.e. vernalization and photoperiod) is critical for determining the adaptive nature of accessions and their use in breeding programmes. According to Kato and Yamashita (1991), genetic variation in these three traits influencing heading date is highest in areas in Iran and Afghanistan and wheat landraces can adapt to these areas by combining different alleles of such traits.

Grain colour is an end-use quality trait, and white-coloured seeds are preferred in Afghanistan. Based on the classification of lines by combining all the three alleles, 40% of the landraces were found to be of white grain type, whereas the remaining were red and amber grain types. Landraces collected in the south-western region tended to have a white *R* allele, whereas those collected in the north-western region had a red *R* allele. When analysing individual loci, the presence of the white allele was prevalent than that of the red allele in all the three homoeologous alleles on chromosome 3 (see online supplementary Table S1). Overall, the white allele (white seed) may have been selected in Afghanistan by the agriculture history.

To determine the potential of KAWLR, diagnostic markers for Lr, Yr, Sr and Fhb were screened. We primarily chose durable rust-resistant genes that were not specific to any races. In the KAWLR set, 17 landraces had positive alleles for the mentioned diseases. The entire set was screened for the Lr34 gene, and a subset of lines was selected based on preliminary field screening (data not shown) and diversity analysis. Four lines were found to be positive for Lr46 and three lines had a Sr-resistant allele. Both Lr34 and Lr46, having a durable resistance mechanism, also showed resistance to multiple diseases (Singh *et al.*, 2011). In Fhb1 screening, only five accessions were found to have resistant alleles and 67 accessions susceptible alleles. Though the gene-specific genotyping revealed that only a few landraces had resistant alleles compared with susceptible ones, it was possible to identify the haplotypes of the known genes. Additionally, our results suggest that new resistance alleles might be present in the accessions evaluated in this study.

Supplementary material

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S1479262114000203>

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