



Evaluation of Wheat Landrace Germplasm for Agronomic Disease Susceptibility and Quality Traits Using Kompetitive Allele-Specific PCR (KASP) Markers

Rekabetçi Alel-Spesifik PCR (KASP) İşaretleyicileri Kullanılarak Buğday Yerel Bitki Germ-Plazmasının Tarımsal Hastalıklara Duyarlılık ve Kalite Özellikleri Açısından Değerlendirilmesi

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EVALUATION OF WHEAT LANDRACE GERMPLASM FOR AGRONOMIC DISEASE SUSCEPTIBILITY AND QUALITY TRAITS USING KOMPETITIVE ALLELE-SPECIFIC PCR (KASP) MARKERS

ABSTRACT

In the study, the genetic variation in agronomic, disease resistance and quality traits of seventy-three wheat landraces from Pakistan was analyzed by using 65 Kompetitive Allele-Specific PCR (KASP) assays. This research was intended to underpin the alleles for economically important genes in wheat landraces. Alleles, *Rht-B1a* and *Rht-B1b* were found in 64% and 30% of the population; while *Rht-D1a* and *Rht-D1b* were identified in 90% and 10% of the population respectively. Alleles responsible for flowering time *Ppd-B1* were detected as sensitive in 46%, and insensitive in 44% of the population while 56 % and 39% insertion and deletions spanning 2Kb distance were observed in *Ppd-D1*. Similarly, 8, 15 and 2 assays were performed to study the genetic variation in vernalization, yield and drought tolerance. About 18% of the landraces were found with *TaDREB_B1a* allele which is drought tolerant. Unfortunately, more than eighty percent of wheat landraces were found to be susceptible to leaf rust confirmed with six assays. In addition, more than 90% of landraces were susceptible to stripe rust, stem rust and *Fusarium* head blight. Identified novel alleles from the current panel of wheat landraces can be used for marker-assisted as well genomic selection by breeding programs.

Keywords: Agronomic Traits, Disease Resistance, Drought Tolerance, *Triticum* spp.



REKABETÇİ ALEL-SPESİFİK PCR (KASP) İŞARETLEYİCİLERİ KULLANILARAK BUĞDAY YEREL BİTKİ GERM-PLAZMASININ TARIMSAL HASTALIKLARA DUYARLILIK VE KALİTE ÖZELLİKLERİ AÇISINDAN DEĞERLENDİRİLMESİ

ÖZ

Çalışmada, Pakistan'daki yetmiş üç yerel buğday çeşidinin agronomik, hastalık direnci ve kalite özelliklerindeki genetik varyasyon, 65 Rekabetçi Alel Spesifik PCR (KASP) tahlili kullanılarak analiz edilmiştir. Bu araştırmanın amacı, yerel buğday çeşitlerinde ekonomik açıdan önemli genlere ilişkin alelleri belirlemektir. Aleller, *Rht-B1a* ve *Rht-B1b* popülasyonun %64 ve %30'unda bulunmuştur; *Rht-D1a* ve *Rht-D1b* sırasıyla popülasyonun %90 ve %10'unda tanımlanmıştır. Çiçeklenme zamanından sorumlu olan aleller *Ppd-B1* popülasyonun %46'sında duyarlı,

%44'ünde duyarsız olarak tespit edilirken, Ppd-D1'de 2kb mesafeyi kapsayan %56 ve %39'luk insersiyon ve delesyonlar gözlenmiştir. Benzer şekilde vernalizasyon, verim ve kuraklık toleransındaki genetik çeşitliliği incelemek için 8, 15 ve 2 tahlilleri yapılmıştır. Yerel çeşitlerde yaklaşık %18'i kuraklığa dayanıklı TaDREB_B1a aleli ile bulunmuştur. Ne yazık ki, yerel buğday türlerinin yüzde sekseninden fazlasının yaprak pasına duyarlı olduğu altı testle doğrulanmıştır. Ek olarak, yerel türlerin %90'ından fazlası şerit pası, gövde pası ve Fusarium baş yanıklığına karşı hassas olduğu tespit edilmiştir. Mevcut yerel buğday çeşitleri panelinden tanımlanan yeni aleller, ıslah programlarıyla genomik seçimin yanı sıra işaretleyici destekli olarak da kullanılabilir.

Anahtar Kelimeler: Tarımsal Özellikler, Hastalık Direnci, Kuraklığa Tolerans, *Triticum* spp.



1. INTRODUCTION

DNA marker-assisted selection (MAS) has a significant role in designing the strategy for improving wheat breeding. DNA markers are generally derived from small DNA regions exhibiting sequence polymorphism among in-species individuals. Most of the random DNA markers (RDMs) are independent of phenotype and can be developed for any species. Such markers were successfully used for genetic diversity studies and to map particular traits. In the beginning, the genetic markers were used for gene mapping to find their order along the chromosomes. These markers were used in plant breeding for germplasm characterization, gene isolation, desirable alleles introgression and variety protection. In the past few decades, numerous important genes in wheat have been found by molecular markers like amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), simple sequence repeats (SSR) and diversity array technology markers (DArT) (Liu et al., 2012; Ozturk and Gul., 2020). The main restriction to use the RDM as a diagnostic tool is the linkage between specific RDM and target locus allele which can be broken by genetic recombination. Additionally, these molecular markers were designed for a specific population and were parent-related.

Recent research on structural and functional genomics resulted in the development of functional markers (FM) as these FMs are the basis for developing the diagnostic markers for MAS and gene introgressions (Rasheed and Xia, 2019). FMs are PCR- based markers designed from polymorphic regions within the functional genes which are directly related to the phenotypic variations. Functional markers are considered ideal for precisely diagnosing the allelic variation at on locus all along with the MAS in plant breeding (Boopathi, 2020). FMs can be used in wheat breeding for various reasons such as; 1) for authenticating a cultivar identity, 2)

selection of the parental material for the construction of a segregating population and 3) following the selection of desirable lines. These molecular markers can apply to any type of population without validating the marker and QTL relationship and regardless of the genetic background of the population. Currently, in wheat, there are about one hundred and fifty-seven FMS been documented for more than 100 loci that are involved in adaptability, agronomic characters, disease resistance, grain yield and processing quality (Rasheed and Xia, 2019). The use of FMs will be increased in the future because of the increased identification of genes related to economic traits. Adaptation of wheat to different environments is largely influenced by three groups of genes, viz, growth rate genes, vernalization genes (*Vrn*) and photoperiod genes (*Ppd*) determine the growth habit. The two semi-dwarf genes *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht1*) are responsible for reduced plant height, enhanced harvest index, improved lodging resistance and a consequent rise in grain size. Functional markers for these two genes have been developed to discriminate between wild-type and semi-dwarf alleles of much importance. The functional markers for genes sucrose synthase2 (*Sus2*), cell-wall invertase (*CWI*) and grain width (*GW2*) which are related to TKW had been developed (Ma et al., 2012). The processing quality of bread wheat is greatly influenced by high and low molecular weight glutenins (HMWs and LMWs), gliadins (*Gli-1*), lipoxygenase (*LOX*) activity, phytoene synthase (*PSY*), polyphenol oxidase (*PPO*) activity, zeta-carotene desaturase (*ZDS*) and granule bound starch synthase I (*GBSSI*). These genes were cloned and functional markers were developed to be used in marker-assisted breeding strategies (Liu et al., 2012). Despite all the advantages, screening a large sample size requires a lot of time, cost and skills to optimize functional markers by using routine PCR and gel electrophoresis. To overcome the insufficiencies these FMs for important economic traits in bread wheat are developed into the Kompetitive allele-specific PCR (KASP) which is a high throughput and breeder friendly (Semagn et al., 2014). These KASP assays proved to be forty-five times faster in speed than conventional gel-based PCR markers (Rasheed et al., 2016a). KASP is a uniplex fluorescence-based SNP genotyping technology, which utilizes allele-specific oligo extension and fluorescence resonance energy transfer (FRET) for signal generation (Kumpatla et al., 2012). Because of its low cost, high efficiency, higher specificity and sensitivity it has been extensively used for SNP genotyping studies such as marker-assisted breeding (MAB) for disease tolerance in like leaf and stem rust resistance (Neelam et al., 2013), wheat streak mosaic virus resistance (Tan et al., 2017), pre-harvest sprouting resistance (Cabral et al., 2014), *Fhb1* (Steiner et al., 2017; Su et al., 2018; Singh et al., 2019) and many agronomic traits (Rasheed et al., 2016a).

Modern bread wheat and other food crops had lost their genetic diversity by selective breeding. Therefore the identification and utilization of new genetic resources are needed for developing genetically diverse crops (Rasheed et al., 2018; Kutlu and Sirel, 2019). Different genetic resources like landraces are the most sig-

nificant means of improving genetic diversity and introgression of beneficial alleles in wheat (Mujeeb-Kazi et al., 2013). A great level of genetic diversity was observed in A. E. Watkins collections (Wingen et al., 2014). A series of useful genes have been identified in this collection as *Lr52* (Hiebert et al., 2005) and *Yr47* (Bansal et al., 2011). Using wheat landraces for crossing and introgression of useful traits is an attractive option in comparison with the complex approaches.

The main objectives of this study were to recognize (1) allelic variations at important loci regarding yield, processing quality and adaptability in wheat landraces of Pakistan and (2) identification of genotypes with desirable haplotype for favorable alleles.

2. MATERIALS AND METHODS

Seventy-three wheat landraces belonging to different areas of Pakistan were obtained from the gene bank of Plant Genetic Resource Institute (PGRI), National Agriculture Research Center (NARC), Islamabad. DNA extraction was done by using a modified CTAB method (Rasheed et al., 2016a). Sixty-five KASP markers utilized by Rasheed et al. (2016a) were used for exploring important economic loci related to adaptability, quality and various agronomic traits. The primer sequences and amplification conditions of each gene are described in the table S1. Each KASP marker is composed of two allele-specific forward primers and a common reverse primer. Allele-specific primers contain the FAM (5'GAAGGTGACCAAGTTCATGCT 3') and HEX (5' GAAGGTCGGAGTCAACGGATT 3') tails and along with the targeted SNP at the 3' end. The 3µl reaction mixture was composed of 10-20 ng/µl of dry DNA, 3µl of 1X KASP master mix and 0.056 µl of primer mixture (containing 100 µM each primer).

The following cyclic conditions were used: hot start for 15min at 94°C, 10 touchdown cycles of the 20s at 95°C, for 25s at touch down 65 °C (decreasing -1 per cycle), 30 additional cycles of annealing (for 10s at 95 °C; for 60s at 57°C). The extension step was excluded as the amplicon size is lesser than 120bp. Fluorescence detection of reactions was used to take readings at 40°C for the 30s.

Genotypes with the corresponding alleles were statistically analyzed with respect to their phenotypic traits using pair-wise t-test at five percent level of significance.

Table S1. A supplementary table of trait and markers with primer sequence used in this study

Trait	Marker	Primer sequence (5'-3')	Expected fragment size
Leaf rust, stripe rust	cssfr1	L34DINT9F: TTGATGAAACCAGTTTTTTTCTA L34PLUSR: GCCATTTAACATAATCATGATGGA	517
	cssfr2	L34DINT9F: TTGATGAAACCAGTTTTTTTCTA L34MINUSR: TATGCCATTTAACATAATCATGAA	523
	cssfr3	Lr34DINT9F: TTGATGAAACCAGTTTTTTTCTA Lr34PLUSR: GCCATTTAACATAATCATGATGGA csLV34F: GTTGGTTAAGACTGGTGATGG csLV34R: TGCTTGCTATTGCTGAATAGT	517+150 229
	cssfr4	Lr34DINT9F: TTGATGAAACCAGTTTTTTTCTA Lr34MINUSR: TATGCCATTTAACATAATCATGAA csLV34F: GTTGGTTAAGACTGGTGATGG csLV34R: TGCTTGCTATTGCTGAATAGT	150 523+229
	cssfr5	Lr34DINT9F: TTGATGAAACCAGTTTTTTTCTA Lr34MINUSR: TATGCCATTTAACATAATCATGAA Lr34SPF: GGGAGCATTATTTTTTCCATCATG Lr34DINT13R2: ACTTTCCTGAAAATAATACAAGCA	751 523
	cssfr6	cssfr6_f: CTGAGGCACTCTTTCCTGTACAAAG cssfr6_r: GCATTCAATGAGCAATGGTTATC	652 649
	cssfr7	cssfr7_f: GCGTATTGTAATGTATCGTGAGAG cssfr7_r: CATAGGAATTTGTGTGCTGTCC	247 214
	Sus2-SNP -185/589H2	Sus2-SNP-185: TAAGCGATGAATTATGGC Sus2-SNP-589H2: GGTGTCCTTGAGCTTCTGG	423
	Sus2-SNP -227/589L2	Sus2-SNP-227: CTATAGTATGAGCTGGATCAATGGC Sus2-SNP-589L2: GGTGTCCTTGAGCTTCTGA	381
	Hap-6A-P1	Forward: CGTTACCTCTGGTTTGGGTGTCGTG Reverse: CACCTCTCGAAAATCTTCCCAATTA	949
	Hap-6A-P2	Forward: GAGAAAGGGCTGGTGCTATGGA Reverse: GTAACGCTTGATAAACATAGGTAAT	418
	CWI22	Forward: GGTGATGAGTTCATGGTTAAT Reverse: AGAAGCCCAACATTAAATCAAC	402
	CWI21	Forward: GTGGTGATGAGTTCATGGTTAAG Reverse: AGAAGCCCAACATTAAATCAAC	404
Abiotic stress tolerance	*	P18F: CCCAACCCAAGTGATAATAATCT P18R: TTGTGCTCCTCATGGGTACTT	717
Grain hardness	<i>Pin-a</i>	Forward: TCAACATTTCGTGCATCATCA Reverse: CTTTCATTCGTCAGAGTTCCAT	436
	<i>Pin-b</i>	Forward: ATGAAGACCTTATTCCTCCTA Reverse: CTCATGCTCACAGCCGCC	250
		Forward: ATGAAGACCTTATTCCTCCTA Reverse: CTCATGCTCACAGCCGCT	250
Starch property	<i>Wx-B1</i>	Forward: CTGGCCTGCTACCTCAAGAGCAACT Reverse: CTGACGTCCATGCCGTTGACGA	425

Bread and noodle making Quality		BDFL: CTGGCCTGCTACCTCAAGAGCAACT BRC1: GGTTGCGGTTGGGGTCGATGAC BFC: CGTAGTAAGGTGCAAAAAAGTGCCACG BRC2: ACAGCCTTATTGTACCAAGACCCATGTGTG	778 668
	<i>gluA3aF</i>	AAACAGAATTATTAAAGCCGG	529
	<i>gluA3aR</i>	GGTTGTTGTTGTTGCAGCA	
	<i>gluA3bF</i>	TTCAGATGCAGCCAAACAA	894
	<i>gluA3bR</i>	GCTGTGCTTGATGATACTCTA	
	<i>gluA3cF</i>	AAACAGAATTATTAAAGCCGG	573
	<i>gluA3cR</i>	GTGGCTGTTGTGAAAACGA	
	<i>gluA3dF</i>	TTCAGATGCAGCCAAACAA	967
	<i>gluA3dR</i>	TGGGGTTGGGAGACACATA	
	<i>gluA3eF</i>	AAACAGAATTATTAAAGCCGG	158
	<i>gluA3eR</i>	GGCACAGACGAGGAAGGTT	
	<i>gluA3fF</i>	AAACAGAATTATTAAAGCCGG	552
	<i>gluA3fR</i>	GCTGCTGCTGCTGTGTAAA	
	<i>gluA3gF</i>	AAACAGAATTATTAAAGCCGG	1345
	<i>gluA3gR</i>	AAACAACGGTGATCCAATAA	
	<i>gluB3aF</i>	CACAAGCATCAAAACCAAGA	1095
	<i>gluB3aR</i>	TGGCACACTAGTGGTGGTC	
	<i>gluB3bF</i>	ATCAGGTGTAAAAGTGATAG	1570
	<i>gluB3bR</i>	TGCTACATCGACATATCCA	
	<i>gluB3cF</i>	CAAATGTTGCAGCAGAGA	472
	<i>gluB3cR</i>	CATATCCATCGACTAAACAAA	
	<i>gluB3dF</i>	CACCATGAAGACCTTCCTCA	662
	<i>gluB3dR</i>	GTTGTTGCAGTAGAACTGGA	
	<i>gluB3eF</i>	GACCTTCCTCATCTTCGCA	669
	<i>gluB3eR</i>	GCAAGACTTTGTGGCATT	
	<i>gluB3fF</i>	TATAGCTAGTGCAACCTACCAT	812
	<i>gluB3fR</i>	CAACTACTCTGCCACAACG	
	<i>gluB3gF</i>	CCAAGAAATACTAGTTAACTAGTC	853
	<i>gluB3gR</i>	GTTGGGGTTGGGAAACA	
	<i>gluB3hF</i>	CCACCACAACAAACATTAA	1022
	<i>gluB3hR</i>	GTGGTGGTTCTATACAACGA	
	<i>gluB3iF</i>	TATAGCTAGTGCAACCTACCAT	621
	<i>gluB3iR</i>	TGGTTGTTGCGGTATAATTT	
	<i>gluB3befF</i>	GCATCAACAACAAATAGTACTAGAA	750
	<i>gluB3befR</i>	GGCGGGTCACACATGACA	

3. RESULTS

Total sixty-five KASP markers were used to describe the allelic variation in the diversity panel of seventy-three wheat landraces from Pakistan. The results are described for three groups of genes; genes responsible for desirable agronomic traits, disease resistance and good quality. These loci underpin the agronomic traits like plant stature, flowering time, drought adaptability, end-use quality, grain size and weight, grain color and disease resistance.

3.1. Allelic Variation in Genes for Agronomic Traits

Two KASP related to *Rht* the green revolution gene identified four haplotypes related to two *Rht* loci i.e. *Rht-B1* and *Rht-D1*. The wild-type *Rht-B1a* allele was found in 64.2% and *Rht-B1b* associated with short stature was found in 30% of the population. Similarly, *Rht-D1a* which is associated with tall stature was found predominant with a frequency of 89.6% and *Rht-D1b* was found in 10% of the population. This allele is associated with short stature. Details of all FMs related to various agronomic traits are given in Table 1.

Table 1. Allele frequency of functional genes related to the various agronomic trait in diversity panel derived from wheat landraces.

Trait	Assay	Gene	Alleles	Frequency%
Plant height	Rht-B1_SNP	<i>Rht-B1</i>	<i>Rht-B1a</i>	*64%
			<i>Rht-B1b</i>	30%
	Rht-D1_SNP	<i>Rht-D1</i>	<i>Rht-D1a</i>	***89%
			<i>Rht-D1b</i>	6.5%
Flowering time	TaPpdBJ003	<i>Ppd-B1</i>	Sensitive	46%
			Insensitive	44%
	TaSNPppdB1-10	<i>Ppd-B1</i>	insensitive-(CS)	***94%
			Sensitive	1%
	TaSNPppdB1-4	<i>Ppd-B1</i>	Paragon-type	***98%
			Chayenne type	1%
	TaSNPppdB1-5	<i>Ppd-B1</i>	Paragon-type	***94%
			Chayenne type	1%
	TaPpdDD002	<i>Ppd-D1</i>	Sensitive	***95%
			Loss of function	0.47%
	TaPpdDD001	<i>Ppd-D1</i>	2kb Ins	56%
			cadenza type	***90
	TaELF3-B1	<i>TaELF3-B1</i>	wild type	1%
			A(insertion)	58%
	TaELF3-D1	<i>TaBradi2g14790</i>	T(Deletion)	24%

	TaELF3-D1	<i>TaELF3-D1-1</i>	Wild type	**71%
			Savanah type	2.8%
	TaELF3-D1-2	<i>TaELF3-D1</i>	Wild type	***95%
			Savanah type	1.8%
	PRR73A1-9IND	<i>TaPRR73-A1</i>	<i>Hap-II</i>	**77%
			<i>HapI</i>	18.8%
	PRR73B1-4558	<i>TaPRR73-B1</i>	<i>HapI</i>	***83%
			<i>Hap-II</i>	14%
			Short vernalisation	42%
	Vrn-D1-D1a_A	<i>Vrn-D1</i>	Winter	*62%
			Spring	37%
	VrnD3_SNP751	<i>Vrn-D3</i>	Jagger-type	***96%
			2174-type	1%
Yield	TaSus2-2B_SNP	<i>TaSus2</i>	<i>Hap-L</i>	54%
			<i>Hap-H</i>	37%
	TaGS-D1_SNP	<i>TaGS-D1</i>	<i>TaGS-D1a</i>	***82%
			<i>TaGS-D1b</i>	16.9%
	TaCKX-D1-IND	<i>TaCKX-D1</i>	<i>TaCKX-D1a</i>	***83%
			<i>TaCKX-D1b</i>	0.75%
	TaGASR-IND	<i>TaGASR-A1</i>	<i>H1g</i>	***83%
			<i>H1c</i>	10%
	TaCwi-A1	<i>CW1-CIMMYT</i>	<i>TaCwi-A1a</i>	54%
			<i>TaCwi-A1b</i>	45%
	TaCwi-4A	<i>CW14A_SNP</i>	<i>Hap-4A-C</i>	***80%
			<i>Hap-4A-T</i>	11%
	TaMoc-2433	<i>TaMoc-7A</i>	<i>Hap-L</i>	***94%
			<i>Hap-H</i>	0.019%
	TaGW2-6A	<i>GW2-CIMMYT</i> <i>GW2-6B</i>	<i>TaTGW2-A1b</i>	***92%
			<i>TaTGW2-A1a</i>	0.94%
	Sus2-2A-20SNP	<i>TaSus2-2A</i>	<i>Hap-G</i>	*73%
			<i>Hap-A</i>	18%
	Sus1-7B-2932IND	<i>TaSus1-7B</i>	<i>Hap-T</i>	*69%
			<i>Hap-C</i>	24%
	TGW6-1050-AL1	<i>TaTGW6-A1</i>	<i>TaTGW6-A1a</i>	***95%
			<i>TaTGW6-A1b</i>	4.7%
	TEF7A_547	<i>TEF-7A</i>	<i>Hap-7A-1, 2</i>	***93%
			<i>Hap-7A-3</i>	10%
	TEF7A1_606	<i>TEF-7A</i>	<i>C</i>	*67%
			<i>A</i>	2.8%
	GS5-2334-SNP	<i>TaGS5-A1</i>	<i>TaGS5-A1a</i>	58%
			<i>TaGS5-A1b</i>	39%
	TGW6-4A	<i>TGW6-4A</i>	<i>TaTGW6-b</i>	***99%
			<i>TaTGW6-a</i>	1%

Drought tolerance	TaDreb_SNP	TaDreb_B1	TaDREB_B1b	**74%
			TaDREB_B1a	18%
	fehw3_SNP	lfehw3	Westonia-type	54%
			Kauz type	45%

*, **, and *** indicate significant differences between the phenotypes of the two alleles for the respective trait, with p-values < .05, < .01, and < .001, as determined by a t-test comparing the phenotypes of the two alleles.

KASP assay was used to validate allelic variation for photoperiod response genes; *Ppd-1* loci i.e. *Ppd-B1* and *Ppd-D1*. Six KASP assays were used at this locus two for *Ppd-D1* and four for *Ppd-B1*. At *Ppd-B1* locus two assays identified a paragon type insensitive allele in 98% of the population. The other two identified an insensitive-CS type allele in 94% and a paragon type insensitive in 98% of the population. At *Ppd-D1* KASP assays had identified 5bpInDel and a wild type allele with a sensitive phenotype associated.

Eleven KASP assays were utilized to distinguish at *VRN-D1* loci. This includes six representing polymorphism at *Vrn-A1*, three at *Vrn-B1* and two at *Vrn-D1* locus. *Vrn-A1* separates between the winter and spring type allele. Assays Vrn-A1_9K0001, Vrn-A1b-Marq and vrn_5Aprm.K.38 each identified winter type allele as predominant one. Vrn1_new and Vrn-A1.Y.14418 identified jagger (short vernalization) and 2147-type allele (long vernalization) at this locus. These identified 2147-type allele and jagger type as predominate alleles respectively. Exon7_C/T_ Vrn-A1 distinguishes between Claire type (early flowering) and Hereward type allele. It was witnessed that Claire-type allele was predominant. Three KASP assays used for *Vrn-B1* locus i.e. *Vrn-B1_A*, *Vrn-B1_B* and *Vrn-B1_C*, all distinguishing between winter and spring type habit. *Vrn-B1_B* and *Vrn-B1_C* both identified winter allele as a predominant one whereas for *Vrn-B1_A* the spring type allele was predominant with a frequency of 96%. At *Vrn-D1* the assays Vrn-D1-D1a_A identified winter type allele as predominant with a frequency of 66%. At the same locus, VrnD3_SNP751 distinguished jagger type allele as a predominant one at this locus.

Nineteen KASP assays related to TKW which is an important yield-related trait was developed. Alleles with low TKW were found predominant for KASP markers like TaSus2-2B_SNP (58%), TaCKX-D1(83%), TaGASR (89%), TaGW2-6A (98%), Sus2-2A-20SNP (78%), TEF7A1_606 (95%), GS5-2334-SNP (62%) and TGW6-4A (99%). Whereas few markers like TaGS-D1 (77%), TaCwi (58%), Sus1-7B-2932IND, TGW6-1050-AL1 (95%) and TEF7A1_606 (71%) have alleles with high TKW as predominant one. A favorable allele was present in high frequency (85%) for CWI_4A_1523. KASP assay for TaMoc-A1 gene related with spike number has low spike number allele (Hap L) as most predominant one (94%).

Two KASP assays for drought-tolerant genes *TaDreb-B1* and *l-feh-w3* were used. The former has a drought-tolerant allele (74%) and later has westonia type (54%) as a predominant.

3.2. Allelic Variation in Genes for Disease Resistance

In this category, six KASP assays were utilized for the presence of leaf rust resistance genes e.g. *Lr9*, *Lr14*, *Lr34*, *Lr9*, *Lr67* and *Lr68* (Table 2). KASP markers have identified the susceptible alleles in the frequency of 99%, 66%, 88%, 99%, 89% and 89% respectively. Results indicated that these landraces were very much susceptible to leaf rust as a wide majority of the landraces were susceptible. Two KASP *Sr36*/*Pm6_8085* and *Sr2_ger9 3p* assays were applied for the determination of *Sr2* genes and one for *Sr36* genes. Both of these assays identified 94% and 86% susceptible alleles respectively. KASP assay *snp3BS-8* identified *fusarium* head blight susceptible allele in 96% frequency.

Table 2. Allele frequency of genes related to various diseases trait in diversity panel derived from wheat landraces.

Trait	Assay	Gene	Alleles	Frequency %
Leaf rust	Lr9_SNP	<i>Lr9</i>	Susceptible	***99%
			Resistant	1%
	ubw14	<i>Lr14</i>	Susceptible	*67%
			Resistant	33%
	Lr34_TCCIND	<i>Lr34</i>	Susceptible	***88%
			Resistant	5%
	Lr9_SNP	<i>Lr9</i>	Susceptible	***99%
			Resistant	1%
	CSTM4_67G	<i>Lr67-</i>	Susceptible	***89%
			Resistant	10%
Stripe rust	Lr68-2	<i>Lr68</i>	Susceptible	***89%
			Resistant	10%
	Yr15-R5	<i>Yr15</i>	Susceptible	***99%
			Resistant	1%
Stem rust	Yr15-R8	<i>Yr15</i>	Susceptible	***89%
			Resistant	9%
	<i>Sr36</i> / <i>Pm6_8085</i>	<i>Sr36</i>	Susceptible	***94%
			Resistant	6%
<i>Fusarium</i> Head blight	<i>Sr2_ger9 3p</i>	<i>Sr2</i>	Susceptible	***86%
			Resistant	13%
	<i>snp3BS-8</i>	<i>Fhb1</i>	Susceptible	***96%
			Resistant	3%

*, **, and *** indicate significant differences between the phenotypes of the two alleles for the respective trait, with p-values < .05, < .01, and < .001, as determined by a t-test comparing the phenotypes of the two alleles.

3.3. Allelic Variation in Genes for Quality

Forty-five KASP assays were used to analyze allelic variations in loci that affecting end-use quality, including grain color, grain hardness, grain protein, waxiness, polyphenol oxidase, phytoene synthase, zeta-carotene, lipoxygenase and avenin like protein. Three KASP assays PPO-D1, PPOB2c_827 and PPOD2_98IND_R have low polyphenol oxidase allele as predominant ones with the frequencies of 50%, 99% and 94%. The remaining two assays showed PPOA1 (25%) and A2a (95%) related to high PPO content were predominant. Four KASP assays were done for four yellow pigment content (YPC) related genes, two for phytoene synthase gene (PSY) and two for the zeta-carotene gene (Zds). For phytoene synthase, KASP assay PsyA1-IND and PsyB1c identified Psy-A1a (high YPC) and Psy-B1a or b (low YPC) as predominant genes. For Zds genes related KASP assays, two alleles *TaZds-A1b* (66%) and *TaZds-D1b* (95%) were predominant. For lipoxygenase activity, the predominant allele *Lox-B1a* (96%) showed high lipoxygenase activity. KASP marker for phytoene desaturase enzyme activity identified *Tapds-B1a* allele in higher frequency (96%) related with high enzyme activity. KASP assay for enzyme lycopene cyclase identified *TaLYC-B1b* as a predominant allele showing lower YPC (Table 3).

Table 3. Allele frequency of functional genes related to various quality attributes trait in diversity panel derived from wheat landraces.

Trait	Assay	Gene	Alleles	Frequency%
Polyphenol Oxidase	PPOA1_1569	<i>Ppo-A1</i>	<i>Ppo-A1a</i>	25%
			<i>Ppo-A1b</i>	9%
	PPO-D1_SNP	<i>PPO-D1</i>	<i>Ppo-D1a</i>	47%
			<i>Ppo-D1b</i>	46%
	PPOA2b_230	<i>PPO2A-2A</i>	A2a	***95%
			A2c	2.8%
	PPOB2c_827	<i>PPO2-2B</i>	B2c	***93%
			B2a, b	3.7%
Phytoene synthase	PPOD2_98IND_R	<i>PPO2-2D</i>	D2b	***94%
			D2a	0.94%
	PsyA1-IND	<i>Psy-A1</i>	<i>Psy-A1a</i>	***81%
			<i>Psy-A1b</i>	15%
	Psy-B1c-SNP	<i>Psy-B1</i>	<i>Psy-B1a or b</i>	***96%
Zeta carotene gene			<i>Psy-B1c</i>	3.7%
	Zds-A1-SNP	<i>Zds-A1</i>	<i>TaZds-A1a</i>	***66%
			<i>TaZds-A1b</i>	21%
	ZDS-D1_SNP	<i>ZDS-D1</i>	<i>TaZDs-1b</i>	***95%
Lipoxygenase Activity			<i>TaZDs-D1a</i>	0.94%
	LoxB1_SNP	<i>LoxB1</i>	<i>Lox-B1a</i>	***96%
			<i>Lox-B1b</i>	0.94%

Phytoene desaturase	Pds-B1_2002	<i>PDS-B1</i>	<i>TaPds-B1a</i>	***90%
			<i>TaPds-B1b</i>	1.8%
Lycopene	LYCB1_3765	<i>Lyce-B1</i>	<i>TaLyc-B1b</i>	45%
			<i>TaLyc-B1a</i>	38%
Grain hardness	Pina-D1_INS	<i>Pina-D1</i>	<i>Pina-D1b</i>	55%
			<i>Pina-D1a</i>	36%
	Pinb-D1_INS	<i>Pinb-D1</i>	<i>Pinb-D1b</i>	*64%
			<i>Pinb-D1a</i>	30%
	Pinb2-IND	<i>Pinb2-v2</i>	<i>Pinb-B2a</i>	***89%
			<i>Pinb-B2b</i>	8.4%

*, **, and *** indicate significant differences between the phenotypes of the two alleles for the respective trait, with p-values < .05, < .01, and < .001, as determined by a t-test comparing the phenotypes of the two alleles.

For characterizing grain hardness three KASP assays were used Pina-D1_INS, *Pinb-D1_INS* and *Pinb2-v2-3*. At *Pina-D1* and *Pinb-D1* locus, the predominant alleles were *Pinb-D1b* associated with hard grain texture in the frequencies of 51% and 64% respectively. KASP marker for Wx-B1 had identified Wx-B1a allele as the predominant one with a frequency of 62%.

4. DISCUSSION

The genetic architecture of the Pakistani wheat landraces was studied by using HTP KASP markers for the functional genes of agronomic traits, good quality and disease resistance. The present study is helpful to identify the alleles responsible for favorable breeding traits which will be subjected to selection sweep and the selected traits will be integrated in wheat by future breeding programs. Although there is a lot of investigation made for finding the functional genes in wheat yet it has been challenging to apply molecular breeding in wheat (Liu et al., 2012). The main reason is the lack of HTP genotyping platform that can integrate with the wheat breeding for large-sized population without tailoring the flexibility (Rasheed et al., 2018; Rasheed and Xia, 2019). This can be done by HTP genotyping assay such as KASP for genotyping the large population at many loci (Rasheed et al., 2016a). Recently, many studies have been practicing the KASP assay to detect the allelic differences for many functional genes in bread wheat cultivars from Canada (Perez-Lara et al., 2017), China (Rasheed et al., 2016a) and US (Grogan et al., 2016). It has been reported earlier that the desirable introgressions from the wheat wild relatives greatly improve the adaptability, resistance, grain yield and end-use quality (Rasheed et al., 2018).

Plant height is a substantial agronomic trait controlled by *Rht-B1(Rht1)* and *Rht-D1(Rht2)* genes. Both of these were widely identified in bread wheat cultivars as these genes cause reduced height (14-17%), increased resistance to lodging, improved Harvest Index which ultimately leads to higher grain yield (Rasheed et al.,

2016a). The wild-type *Rht-B1a* was found in higher frequencies in this germplasm than *Rht-B1b* which is associated with short stature in this germplasm. *Rht-D1a* which is associated with tall stature was also predominant with the frequencies of 89.6%. This was expected as landraces are not commercially improved cultivars so that those were lacking the genes for short stature. Whereas the modern semi-dwarf wheat cultivars with short height have *Rht-B1b* and *Rht-D1b* genes responsible for not only the decreased height but also provide resistance against lodging and increases the harvest index resulted in increased grain yield (Rasheed et al., 2016a). Previous findings revealed the association of *Rht1* genes with many important traits like heading date, grain yield, plant height, relative water contents, spike length, grains per spike and thousand-grain yield (Würschum et al., 2018). Hence, governing the plant height by selecting proper *Rht* alleles which confer the intended environment will remarkably affect the pure-line breeding but also the wheat hybrid development programs as male tallness is desirable for efficient production of hybrid (Khalid et al., 2019).

The adaptability of wheat to multi environments is mainly affected by three genes group; 1) growth rate genes, 2) vernalization genes (*Vrn*) and 3) photoperiod response genes (*Ppd*). *Vrn-1* and *Ppd-1* have a significant effect the development of floral and the determination of adaptation traits for optimum yield (Boden et al., 2015). Genes that are responsible for *Ppd-1* were located on chromosome 2A (*Ppd-A1*), 2B (*Ppd-B1*) and 2D (*Ppd-D1*). Wheat is classified into two groups depending upon its response to the daylight i.e. 'photoperiod sensitive' and 'photoperiod insensitive' (Zhou et al., 2016). Photoperiod sensitivity is influenced by *Ppd-D1* and *Ppd-B1* and *Ppd-A1* has less effect on it (Grogan et al., 2016). Out of six KASP assays used for *Ppd-B1*, three identified insensitive type alleles as major alleles in the population. At *Ppd-D1* locus KASP assays have identified alleles associated with the sensitive phenotype. Winter wheat cultivars in the United States, Germany and France were photoperiods sensitive than countries from Southern latitudes were insensitive. In Europe up to 35% of yield advantages are the result of photoperiod insensitivity in bread wheat. The *Ppd-1* was also associated with various other economical features like grain yield, thousand kernel yield and spike length and hence can play role in grain yield determination (Khalid et al., 2019). Vernalization in wheat is controlled majorly by *Vrn-1* genes. The dominant allele at any of *Vrn-A1*, *Vrn-B1* and *Vrn-D1* confers a spring growth habit. Among these *Vrn-A1* has the greatest effect of inhibiting vernalization, followed by *Vrn-D1* and *Vrn-B1* which have the lowest effect. The growth habit of wheat landraces is spring same as Pakistani bread wheat cultivars. Vernalization is a major flowering signal which remains repressed until cold-induced. Afghan wheat landraces collected by Dr. Kihara also revealed the presence of winter-type alleles in higher percentages (Manickavelu et al., 2014). The previously frequent spring-type habit was reported at *Vrn-B1* in 64% and *Vrn-D1* in 61% (Iqbal et al., 2011).

The genetic architecture of grain yield is poorly understood as it is a complex quantitative with low heritability and is significantly affected by the environment. This makes it difficult to be manipulated and incorporated into breeding programs. Previously reported KASP markers developed for functional genes related to grain yield were based on grain size and weight as it is significantly influenced by these two components (Rasheed et al., 2016a). Most of the KASP assays identified alleles related to low TKW and few of them have identified alleles related to high TKW. Cell wall invertase (CWI) enzyme is also associated with crop yield especially in the stressed environment. Functional markers for this gene were developed by (Hou et al., 2014). A favorable allele for this locus was present in higher frequencies in our germplasm. Previous screenings of landraces and cultivars from Pakistan have highlighted their potentials for using many yield-related genes to increase grain yield (Rasheed et al., 2016b). KASP markers have also identified alleles related to low spike numbers as the predominant ones. A favorable allele for this locus was identified in low frequencies in the cultivar panel by (Rasheed et al., 2016a).

Two assays for drought tolerance have indicated two drought-tolerant alleles as predominant alleles in these landraces. One of these alleles is Westonia type which was found to be related to higher TKW under drought (Zhang et al., 2015). So these landraces can be used for improving the drought tolerance in bread wheat by breeding programs aligns with the molecular approach.

Polyphenol oxidase (PPO) is an enzyme responsible for an undesirable brown color of the wheat end products, especially Asian noodles. That is the reason for its low PPO activity is desirable (Liu et al., 2012). Molecular markers have been developed for PPO genes on chromosomes 2A and 2D to screen varieties with low PPO activity. Allelic variations at *Ppo-A1* and *Ppo-D1* are associated with PPO activity. Similarly, *Ppo-D1* is classified into *Ppo-D1a* and *Ppo-D1b* alleles associated with low and high PPO activity (Hystad et al., 2015). *Ppo-A1a* and *Ppo-A1b* are related to high and low PPO activity respectively. In this germplasm, three KASP assays had shown *Ppo-D1a*, *B2c* and *D2a* as predominant alleles associated with low PPO activity. The remaining two had shown *PPoA1* and *A2a* in higher frequencies related to high PPO activity. FMs have previously been used to select wheat varieties with low PPO activity.

The yellow color of wheat is due to carotenoids. Phytoene synthase (*Psy1*) and zeta-carotene desaturase (*Zds1*) are major enzymes in the biosynthesis of carotenoids (Dong et al., 2012). Three groups of phytoene synthase located on chromosomes 7 and 5 are *Psy1*, *Psy2* and *Psy3*. The *Psy-A1a* and *Psy-A1b* are associated with high and low grain carotenoid contents respectively. Four allelic variations for *Psy-B1* gene have been reported i.e. a, b, c, and d. Low grain carotenoid content is associated with *Psy-B1a* and *Psy-B1b*. While high grain carotenoid is associated with *Psy-B1c* (He et al. 2009). *Psy-B1a* or b (low YPC) was present in higher percent-

ages in this germplasm. For *Zds* a large percentage of germplasm have *TaZds-D1b* associated with low YPC. Lipoxygenase (LOX) activity also has a great influence on the color and processing qualities of wheat-based products. FMs have revealed *Lox-B1a* as the main allele in the germplasm associated with high LOX activity. For phytoene desaturase, the enzyme allele identified in higher frequencies was related to higher yellow color.

Grain hardness has a noticeable effect on end-use quality and milling. This is mostly determined by *Pina-D1* and *Pinb-D1*, which encode puroindoline a and puroindoline b proteins, respectively. Hard texture generates coarse flour in which more water is absorbed while the soft endosperm results in small particles and smaller particles. Hard grains are suitable for bread making whereas cookies and biscuits are made from soft grains. Chromosome 5D contains the genes for *Pina-D1* and *Pinb-D1*. These genes may be wild-type and mutated ones. Wild type allele includes 'a' which is associated with hard texture while the alleles 'b, c and d' are mutated ones related with soft texture (Mohammadi et al., 2013). This germplasm contained higher frequencies of *Pinb-D1b* associated with hard textured grains. *Pinb-D1b* is associated with higher flour yield, good crumb grain scores, increased loaf volume and decreased ash (Hogg et al., 2005). Characterization of Watkin's collections revealed that *Pinb-D1b* was present in lesser percentages than *Pina-D1b* (Qamar et al., 2014). Waxy protein also called granule bound starch synthase 1 (GBSS) is an important quality protein. This enzyme has an important role in amylose synthesis in the endosperm tissue. Noodle quality is determined by amylose content. Wild type waxy protein is called *Wx-B1b* and one which carries deletion is called *Wx-B1a*. The germplasm contains *Wx-B1a* in the majority. It has been reported earlier that this Waxy gene in wheat had a direct association with wheat flour quality and the property of its starches (Vafin et al., 2018).

Yield losses are expected when crops are susceptible to biotic and abiotic stresses. Biotic stress includes leaf rust (causative agent *Puccinia triticina*), stripe rust (causative agent *P. striiformis*) and stem rust (causative agent *P. graminis*) is the most damaging one (Pretorius et al., 2000). Landraces were screened *Lr14*, *Lr34*, *Lr9*, *Lr67* and *Lr68* by using KASP markers. Our study indicated that the majority of landraces were susceptible to all of these genes. Similarly, landraces were also found to be susceptible to stem rust when screened for *Sr2* genes and *Sr36*. *Sr2* is one of the identified durable, race non-specific adult plant stem rust resistance genes in wheat. It was transferred from tetraploid emmer wheat (*Triticum dicoccum Schronk*) into common wheat (*T. aestivum* L.) 'Marquis' in 1920 (McFadden 1930) and is still found to be effective against all stem rust pathotypes of wheat. *Sr2* response is described as partial resistance and is only expressed at the adult plant stage. Functional markers for *Lr34/Yr18/Pm38* were used to screen 151 Chinese wheat landraces. Results identified that one-quarter of these landraces were very sensitive to stripe rust in the field (Wu et al., 2010).

KASP markers has gained considerable attention in agricultural genomics, study by Steele et al. (2025) developed thousands KASP markers for diverse breeding applications in rice. For *Fusarium* head blight resistance in wheat (Li et al. 2024), validating quantitative trait loci (QTLs) and streamlining marker-assisted selection (MAS). In relation to climate resilience, KASP markers were deployed to identify drought-tolerant alleles in maize, aiding breeding programs (Rauf et al. 2024). Cost effectiveness of these KASP markers along with their robot-aided multiplexing has improved the functionality as well as accuracy for the future demands.

5. CONCLUSIONS

Our research exploited the allelic variations in 73 bread wheat landraces from Pakistan for stress tolerance (biotic and abiotic), quality and agronomic traits by sixty-five using KASP markers. The economically important alleles for all the above-mentioned traits in the bread wheat landraces were identified. Unluckily the selected wheat landraces were susceptible to all kinds of rusts and *fusarium* head blight. The novel alleles identified for drought stress tolerance, quality and agronomic traits will be helpful in MAS by breeding programs to improve its quality and productivity to reach the targeted wheat production.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Ethics

This study does not require ethics committee approval.

Author Contribution Rates

Design of Study: AG (%50), PJ (%20), RA (%15), FM (%15)

Data Acquisition: SSL (%100)

Data Analysis: MJ (%50), HA (%50)

Writing up: SSL (%90), MJ (%10)

Submission and Revision: BTU (%60) MO (%40)

REFERENCES

- Bansal, U.K., Forrest, K.L., Hayden, M.J., Miah, H., Singh, D., Bariana, H.S., 2011. Characterisation of a new stripe rust resistance gene Yr47 and its genetic association with the leaf rust resistance gene Lr52. *Theoretical and Applied Genetics* 122 (8):1461-1466. doi: 10.1007/s00122-011-1545-4
- Boden, S.A., Cavanagh, C., Cullis, B.R., Ramm, K., Greenwood, J., Finnegan, E.J., Trevaskis, B., Swain, S.M., 2015. Ppd-1 is a key regulator of inflorescence architecture and paired spikelet development in wheat. *Nature Plants* 1(2):14016. doi: 10.1038/nplants.2014.16
- Boopathi, N.M., 2020. Marker-assisted selection (MAS). In *Genetic Mapping and Marker Assisted Selection*, 343-388. Springer.
- Cabral, A.L., Mark, C.J., McCartney, C.A., You, F.M., Humphreys, D.G., MacLachlan, R., Pozniak, C.J., 2014. Identification of candidate genes, regions and markers for pre-harvest sprouting resistance in wheat (*Triticum aestivum* L.). *BMC Plant Biology* 14 (1):1-12. doi: 10.1186/s12870-014-0340-1
- Dong, C.H., Xia, X.C., Zhang and, L.P., He, Z.H., 2012. Allelic variation at the TaZds-A1 locus on wheat chromosome 2A and development of a functional marker in common wheat. *Journal of Integrative Agriculture* 11(7):1067-1074. doi:10.1016/S2095-3119(12)60099-9
- Grogan, S.M., Brown-Guedira, G., Haley, S.D., McMaster, G.S., Reid, S.D., Smith, J., Byrne, P.F., 2016. Allelic variation in developmental genes and effects on winter wheat heading date in the US Great Plains. *PLoS One* 11(4):e0152852. doi: 10.1371/journal.pone.0152852
- He, X.Y., He, Z.H., Ma, W., Appels, R., Xia, X.C., 2009. Allelic variants of phytoene synthase 1 (Psy1) genes in Chinese and CIM-MYT wheat cultivars and development of functional markers for flour colour. *Molecular Breeding* 23(4):553-563.
- Hiebert, C., Thomas, J., McCallum, B., 2005. Locating the broad-spectrum wheat leaf rust resistance gene Lr52 (LrW) to chromosome 5B by a new cytogenetic method. *Theoretical and Applied Genetics* 110 (8):1453-1457. doi: 10.1007/s00122-005-1978-8
- Hogg, A.C., Beecher, B., Martin, J.M., Meyer, F., Talbert, L., Lanning, S., Giroux, M.J., 2005. Hard wheat milling and bread baking traits affected by the seed-specific overexpression of puroindolines. *Crop Science* 45(3):871-878. doi:10.2135/cropsci2004.0113
- Hou, J., Jiang, Q., Hao, C., Wang, Y., Zhang, Y., Zhang, X., 2014. Global selection on sucrose synthase haplotypes during a century of wheat breeding. *Plant Physiology* 113.232454. doi: 10.1104/pp.113.232454
- Hystad, S.M., Martin, J.M., Graybosch, R.A., Giroux, M.J., 2015. Genetic characterization and expression analysis of wheat (*Triticum aestivum*) line 07OR1074 exhibiting very low polyphenol oxidase (PPO) activity. *Theoretical and Applied Genetics* 128(8):1605-1615.
- Iqbal, M., Shahzad, A., Ahmed, I., 2011. Allelic variation at the Vrn-A1, Vrn-B1, Vrn-D1, Vrn-B3 and Ppd-D1a loci of Pakistani spring wheat cultivars. *Electronic Journal of Biotechnology* 14(1):1-2.
- Khalid, M., Afzal, F., Gul, A., Amir, R., Subhani, A., Ahmed, Z., Mahmood, Z., Xia, X., Rasheed, A., He, Z. 2019. Molecular characterization of 87 functional genes in wheat diversity panel and their association with phenotypes under well-watered and water-limited conditions. *Frontiers Plant Science* 10:717. doi: 10.3389/fpls.2019.00717
- Kumpatla, S.P., Buyyarapu, R., Abdurakhmonov, I.Y., Mammadov, J.A., 2012. Genomics-assisted plant breeding in the 21st century: technological advances and progress. In *Plant breeding*. InTech. doi: 10.1007/s11032-012-9773-0
- Kutlu, I., Sirel, Z., 2019. Using Linx Tester Method and Heterotic Grouping to Select High Yielding Genotypes of Bread Wheat (*Triticum aestivum* L.). *Turkish Journal of Field Crop*. 24(2):185-194. doi: 10.17557/tjfc.643546
- Li, C., Wu, L., He, X., He, Y., Jiang, P., Ma, J., Singh, P.K., Zhang, X., (2024). Identification and validation of two QTL associated with Fusarium head blight resistance in spring wheat (*Triticum aestivum* L.). *Journal of Integrative Agriculture* doi.org/10.1016/j.jia.2024.12.021
- Liu, Y., He, Z., Appels, R., Xia, X., 2012. Functional markers in wheat: current status and future prospects. *Theoretical and Applied Genetics* 125(1):1-10. doi:10.1007/s00122-012-1829-3
- Ma, D., Yan, J., He, Z., Wu, L., Xia, X., 2012. Characterization of a cell wall invertase gene TaCwi-A1 on common wheat chromosome 2A and development of functional markers. *Molecular Breeding* 29(1):43-52. doi: 10.1007/s11032-010-9524-z
- Manickavelu, A., Jighly, A., Ban, T., 2014. Molecular evaluation of orphan Afghan common wheat (*Triticum aestivum* L.) landraces collected by Dr. Kihara using single nucleotide polymorphic markers. *BMC Plant Biology* 14(1):320. doi:10.1186/s12870-014-0320-5
- McFadden, E.S. 1930. A successful transfer of emmer characters to vulgare wheat. *Journal of the American Society of Agronomy* 22:1020-34.
- Mohammadi, M., Mehrazar, E., Izadi-Darbandi, A., Najafian, G., 2013. Genotype diversity of puroindoline genes (PinA-D1 and PinB-D1) in bread wheat cultivars developed in Iran and CIMMYT. *Journal of Crop Improvement* 27(4):361-375. doi: 10.1080/15427528.2013.775988
- Mujeeb-Kazi, A., Gul Kazi, A., Dundas, I., Rasheed, A., Ogbonnaya, F., Kishii, M., Bonnett, D., Wang, R.R.-C., Xu, S., Chen, P., 2013. Genetic diversity for wheat improvement as a conduit to food security. *Advances in Agronomy* 122:179-258.

- Neelam, K., Brown-Guedira, G., Huang, L., 2013. Development and validation of a breeder-friendly KASPar marker for wheat leaf rust resistance locus Lr21. *Molecular Breeding* 31(1):233-237. doi: 10.1007/s11032-012-9773-0
- Ozturk, M., Gul, A., 2020. Climate Change and Food Security with Emphasis on Wheat: Academic Press.
- Perez-Lara, E., Semagn, K., Chen, H., Ciechanowska, I., Iqbal, M., N'Diaye, A., Pozniak, C., Strelkov, S.E., Hucl, P.J., Graf, R.J., 2017. Allelic variation and effects of 16 candidate genes on disease resistance in western Canadian spring wheat cultivars. *Molecular Breeding* 37(3):23.
- Pretorius, Z.A., Singh, R.P., Wagoire, W.W., Payne, T.S., 2000. Detection of virulence to wheat stem rust resistance gene Sr31 in *Puccinia graminis* f. sp. tritici in Uganda. *Plant Dis.* 84 (2):203-203. doi:10.1094/PDIS.2000.84.2.203B
- Qamar, Z.U., Bansal, U.K., Mei, C., Dong, Alfred, R.L., Bhawe, M., Bariana, H.S., 2014. Detection of puroindoline (Pin-a-D1 and Pin-b-D1) allelic variation in wheat landraces. *Journal of Cereal Science* 60(3):610-616. doi: 10.1016/j.jcs.2014.07.007
- Rasheed, A., Mujeeb-Kazi, A., Chuks Ogbonnaya, F., He, Z., Rajaram, S., 2018. Wheat genetic resources in the post-genomics era: promise and challenges. *Annals of Botany* 121 (4):603-616. doi: 10.1093/aob/mcx148
- Rasheed, A., Wen, W., Gao, F., Zhai, S., Jin, H., Liu, J., Guo, Q., Zhang, Y., Dreisigacker, S., Xia, X., 2016a. Development and validation of KASP assays for genes underpinning key economic traits in bread wheat. *Theoretical and Applied Genetics* 129(10):1843-1860. doi:10.1007/s00122-016-2743-x
- Rasheed, A., Xia, X., 2019. From markers to genome-based breeding in wheat. *Theor. Appl. Genet.* 132(3):767-784. doi: 10.1007/s00122-019-03286-4
- Rasheed, A., Xia, X., Mahmood, T., Quraishi, U.M., Aziz, A., Bux, H., Mahmood, Z., Mirza, J.I., Mujeeb-Kazi, A., He, Z., 2016b. Comparison of economically important loci in landraces and improved wheat cultivars from Pakistan. *Crop Science* 56(1):287-301. doi:10.2135/cropsci2015.01.0015
- Rauf, A., Sher, M. A., Farooq, U., Rasheed, A., Sajjad, M., Jing, R., Khan, Z., Attia, K., Mohammed, A.A., Fiaz, S., Chen, J., Rehman, S. U., 2024. An SNP based genotyping assay for genes associated with drought tolerance in bread wheat. *Molecular Biology Reports* 51(1): 527.
- Semagn, K., Babu, R., Hearne, S., Olsen, M., 2014. Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): overview of the technology and its application in crop improvement. *Molecular Breeding* 33(1):1-14. doi: 10.1007/s11032-013-9917-x
- Singh, L., Anderson, J.A., Chen, J., Gill, B.S., Tiwari, V.K., Rawat, N., 2019. Development and validation of a perfect KASP Marker for *Fusarium* head blight resistance gene Fhb1 in wheat. *Plant Pathology Journal* 35(3):200. doi: 10.5423/PPJ.OA.01.2019.0018
- Steele, K., Quinton-Tulloch, M., Vyas, D., Witcombe, J., 2025. Thousands of trait-specific KASP markers designed for diverse breeding applications in rice (*Oryza sativa*). G3: Genes, Genomes, Genetics, 15(1): jkae251.
- Steiner, B., Zimmerl, S.R.P., Muhl, S., Lemmens, M., Adam, G., Till, B., Schweiger, W., Buerstmayr, H., 2017. Functional identification of the wheat gene enhancing mycotoxin detoxification of the major *Fusarium* resistance QTL Fhb1. 13th International Wheat Genetics Symposium. Tulln, Austria: BOKU-University of Natural Resources and Life Sciences, Vienna.
- Su, Z., Jin, S., Zhang, D., Bai, G., 2018. Development and validation of diagnostic markers for Fhb1 region, a major QTL for *Fusarium* head blight resistance in wheat. *Theoretical and Applied Genetics* 131(11):2371-2380. doi:10.1007/s00122-018-3159-6
- Tan, C.T., Assanga, S., Zhang, G., Rudd, J.C., Haley, S.D., Xue, Q., Ibrahim, A., Bai, G., Zhang, X., Byrne, X., 2017. Development and validation of KASP markers for wheat streak mosaic virus resistance gene Wsm2. *Crop Science* 57(1):340-349. doi: 10.2135/cropsci2016.04.0234
- Vafin, R., Rzhanova, I., Askhadullin, D., Vasilova, N., 2018. Screening of the genotypes of bread wheat (*Triticum aestivum* L.) by the allelic variants of Waxy genes and HMW glutenin subunits. *Acta Agrobotanica* 71(4). doi:10.5586/aa.1746
- Wingen, L.U., Orford, S., Goram, R., Leverington-Waite, M., Bilham, L., Patsiou, T.S., Ambrose, M., Dicks, J., Griffiths, S., 2014. Establishing the AE Watkins landrace cultivar collection as a resource for systematic gene discovery in bread wheat. *Theoretical and Applied Genetics* 127(8):1831-1842. doi:10.1007/s00122-014-2344-5
- Wu, L., Xia, X.C., Zhu, H., Li, S.Z., Zheng, Y.L., He, Z.H., 2010. Molecular characterization of Lr34/Yr18/Pm38 in 273 CIMMYT wheat cultivars and lines using functional markers. *Scientia Agricultura Sinica* 43(22):4553-4561.
- Würschum, T., Leiser, W.L., Langer, S.M., Tucker, M.R., Longin, C.F.H., 2018. Phenotypic and genetic analysis of spike and kernel characteristics in wheat reveals long-term genetic trends of grain yield components. *Theoretical and Applied Genetics* 131(10):2071-2084. doi:10.1007/s00122-018-3133-3
- Zhang, J., Xu, Y., Chen, W., Dell, B., Vergauwen, R., Biddulph, B., Khan, N., Luo, H., Appels, R., Van den Ende, W., 2015. A wheat 1-FEH w3 variant underlies enzyme activity for stem WSC remobilization to grain under drought. *New Phytologist* 205(1):293-305. doi: 10.1111/nph.13030
- Zhou, W., Wu, S., Ding, M., Li, J., Shi, Z., Wei, W., Guo, J., Zhang, H., Jiang, Y., Rong, J., 2016. Mapping of Ppd-B1, a major candidate gene for late heading on wild emmer chromosome arm 2bs and assessment of its interactions with early heading QTLs on 3AL. *PLoS One* 11(2):e0147377. doi: 10.1371/journal.pone.0147377