



## Variability of Q gene locus in Ethiopia bread wheat cultivars and breeding lines

Solomon Berhanu Sharo<sup>1,2,3</sup> Kebebush Desta Dinane<sup>3</sup>, Gizachew Haile Gidamo<sup>\*1,2</sup>

<sup>1</sup>*Biotechnology and Bioprocess Center of Excellence, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia*

<sup>2</sup>*Department of Biotechnology, College of Natural and Applied Sciences, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia*

<sup>3</sup>*Department of Biotechnology, College of Natural and Computational Sciences, Wachemo University, Hossaena, Ethiopia*

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### Abstract

The wheat Q gene, located on the group 5 homologous chromosome, is extremely important for widespread wheat cultivation due to its influence on many morphological traits associated with domestication. To analyze the variability of the Q gene locus, 52 genotypes were grown in pots in Addis Ababa Science and Technology University. Deoxyribonucleic acid (DNA) extraction was done from 20 days old plant by using CTAB buffer. DNA marker designed from the 9<sup>th</sup> intronic region of the Q gene was used to carryout polymerase chain reaction (PCR). The PCR products were separated in agarose gel and six allelic variants from the PCR products were visualized using gel documental system. First, the expected PCR product sizes of 536bp, 665bp and 578bp for locus 5AQ, 5BQ and 5DQ loci, respectively, were observed in 16 bread wheat genotypes. Secondly, amplification of expected PCR product sizes for 5AQ and 5DQ, but no 5BQ were observed for six genotypes. Thirdly, four kinds of length variations in the 9<sup>th</sup> intronic regions for the PCR products were observed for half of the analysed genotypes in 5AQ and 5DQ loci. Furthermore, amplified PCR product for only 5AQ was observed in seven wheat genotypes, but no for 5BQ and 5DQ. The information's obtained on the Q gene profile will contribute towards diversification and knowledge based uses of Q gene in Ethiopian wheat breeding programs, and will be useful in the attempts to develop cultivars with ideal plant architecture and high yield.

\*Corresponding Author: Gizachew Haile ✉ [gizachew.haile@aastu.edu.et](mailto:gizachew.haile@aastu.edu.et)

## Introduction

Wheat (*Triticum aestivum* L.) is one of the most globally grown crops and is the world's second most important cereal crop for food and nutrition (USDA, 2018). It is the most important staple crop in world and accounts 20% of nutritional sources (Khabiri *et al.*, 2012). Wheat provides nearly 55% of carbohydrates, 20% of the daily protein and 21% calories for about 40% of the global population (Khan and Naqvi, 2011). There is an increasing demand for wheat in the global market due to the surge in urban population. In addition, wheat products such as bread, pasta, noodle and biscuits are quick, easy to prepare, and contains substantial amounts of starch, proteins, vitamins and minerals which also made it most preferred food sources in most countries (Shewry and Hey, 2015). Ethiopians also use wheat grain for the preparation of wide range of traditional products like pancake ("injera"), bread, local beer ("tella"), and several other local food items (Nigusse *et al.*, 2015).

Bread wheat (*Triticum aestivum* L.) is an allohexaploid species with an AABBDD genome ( $2n=6x=42$ ) which originated as the result of two separate amphiploidization events. Pasta wheat (*Triticum turgidum* L.,  $2n = 4x = 28$ , AABB), is a tetraploid, which arose as result of hybridization between *Triticum urartu* Tumanian ex Gandylian ( $2n = 2x = 14$ , AA) and an unidentified diploid *Aegilops* species probably *Aegilops spletois* Tausch Coss ( $2n = 2x = 14$ , BB). The D genome of the wheat species is donated by the diploid goat grass *Aegilops tauschii* ( $2n = 2x = 14$ , DD) species (Sleper and Poehlman, 2006).

Wheat is the oldest domesticated grain crops which have been cultivated in Middle East countries (Yadawad *et al.*, 2015). The earliest cultivated forms were diploid genome (AA, einkorn) and tetraploid genome (AABB, emmer) wheat and their genetic relationships indicate that they originated from the south-eastern part of Turkey (Dubcovsky and Dvorak, 2007). Cultivation spread to the Near East by about 9000 years ago when hexaploid bread wheat made its first appearance (Feldman, 2001). Currently, bread

wheat account about 95% of worldwide wheat production and the remaining 5% being tetraploid durum wheat used in pasta and semolina products (Shewry, 2009). It is one of the important cereal crops produced worldwide serving as an important export and strategic commodity (Kumar, 2013). The report on wheat production reveals that world production was 765.4 million metric tons with average yield of 3.48t/ha and it accounts for nearly 30% of global cereal production (FAO, 2019).

In Ethiopia, bread wheat is one of the most important cereal crops in terms of production and consumption (Kelemu, 2017). The national average productivity of wheat is estimated to 2.76 t/ha. It is cultivated on 1.7 million hectares of land and has the total production of 4.83 million tons which remained as low in productivity as compared to the world average yield (CSA, 2019). The key challenges in increasing wheat productivity is the prevalence of biotic (yellow rust, stem rust, septoria, fusarium) and abiotic (acidity, heat and drought) stresses, low adoption of improved technologies, cost and limited availability of inputs, poor infrastructure and marketing systems (Savary *et al.*, 2019; Peng *et al.*, 2011).

Polyploidy and whole genome duplication is important phenomenon in the formation of new plant species or adaptation of a plant species for new habitat. Genes that have been duplicated in polyploid organisms are known to accumulate mutations and eventually become inactive through a process known as non-functionization. In addition, altered expression of coding and noncoding genes, suppression expression in duplicated genes, gaining new functions by means of new expression or new gene structure and genetic and epigenetic interactions all play role in plant adaptation and evolution by polyploidy. They are key players in enabling plant diversification and domestication (Alix *et al.*, 2017).

The wheat domestication-related features such as spike morphology, plant height, inflorescence emergence, and threshability are all under the influence of the wheat domestication (Q) gene.

For extensive cultivation and increased yields, these characteristics are essential (Zhang *et al.*, 2022). The wild wheat q allele confers elongated spikes and hulled grains whereas the domesticated wheat Q gene on chromosome 5A is known to confer subcompact spike, free threshing grains, rachis fragility, plant height and heading time, and grain yield. The wheat domestication gene, encode an APETALA-2 like transcription factor. The domesticated Q allele of 5A was converted from undomesticated q allele through amino acid mutations that substitute amino acids at position 329 (Valine (q) ->isoleucine (Q)). This amino acid substitution may not affect the protein structure (Sormacheva *et al.*, 2014; Debernardi *et al.*, 2017). In addition, mutation at the microRNA172 target sites shown to induce mismatch to microRNA172, that reduced cleaving efficiency and increased Q expression level to result compact spike phenotype (Debernardi *et al.*, 2017). Whereas the 5BQ homeoallele was evolved as pseudo gene after allotetraploidization (Zhang *et al.*, 2011).

The ideal plant architecture correlates with increased grain number and yield. A morphological feature of the ideal plant includes shorter lowermost elongated internode, longer uppermost elongated internode, and longer spike. Development of these morphological features is influenced by copy number variations, especially in the 5Q gene. The three homoeoalleles play directly or indirectly for such phenotype expression. The Q phenotype expression was directly correlated with 5A, but the suppression of splotid phenotype is directly controlled by 5Dq and indirectly by 5Bq (Zhang *et al.*, 2011). Variability in the Q gene locus of Ethiopian wheat cultivars and breeding lines still remain unknown. Furthermore, understanding its functional influence on free-threshing, spike morphology, plant height, floescence emergence and non-fragile rachis features in Ethiopian wheat cultivars needs investigation. Such studies are critical for future selection and breeding to produce cultivars with superior qualities. In this study, variability in the Q gene locus of Ethiopian bread wheat cultivars and breeding lines were studied.

## Materials and methods

### Plant materials

Fifty two (52) bread wheat cultivars and breeding lines were obtained from Kulumsa Agricultural research center (KARC). The materials were planted in a pot at Addis Ababa Science and Technology University, during December 2022-January 2023 for deoxyribonucleic acid (DNA) extraction purpose and allowed to grow up to two leaves stage.

### Genomic DNA extraction and PCR

A twenty day old leaves were used for genomic DNA extraction by using a modified Cetyl trimethyl ammonium bromide (CTAB) extraction buffer (2% (w/v) CTAB, 1.42 M NaCl, 100 mM Tris HCl (pH 8.0), 20 mM EDTA (pH 8.0), and 0.1% Mercaptoethanol (Dellaporta *et al.* 1983)). PCR amplification was performed using PCR thermal cycler in a final volume of 10µl reaction mixture containing 100ng of template DNA, 10 pmole of primer, 2.5 mM of dNTPs, 15 mM of MgCl<sub>2</sub> with 10X PCR buffer and 3 U/µl of Taq polymerase enzyme. The thermocycler was set up to run 35 cycles of PCR amplification at the following temperatures: 30s denaturation at 94°C, 30s of primer annealing at 60°C, and 45s of primer extension at 72°C, followed by a final extension step at 72°C for 10 min. Q gene specific primer designed from 9<sup>th</sup> intronic region of the gene with forward (F) sequence 5`-CGCTGCTCCACCAGCTTACTG-3` and reverse (R) 5`GCCTCTCCTGCACCTGCAC-3` was used to amplify the homoeoalleles from 5AQ, 5BQ and 5DQ (Qi, 2015). The PCR products were separated by electrophoresis in 3% agarose gel and visualized using gel imaging system.

## Results

### Detection of the Q gene homoeoalleles

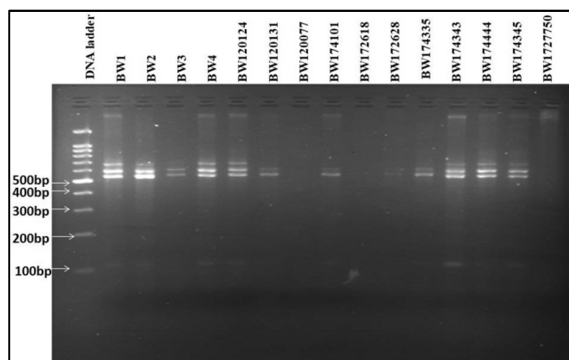
The outcome of the primer tested to predict the presence of the homoeoalleles of 5AQ, 5BQ and 5DQ in the tested entries were provided in table 1. To detect the Q gene loci, gene specific primer were developed from the ninth(9<sup>th</sup>) intronic region showing length variations in 5AQ, 5BQ and 5DQ loci (Qi, 2015). Single primer was used to identify the Q gene homoeoalleles present in the tested genotypes.

At least one of the homoeoallele was amplified from each entries and the Q gene homoeoalleles were considered to be present if the primer amplified 536bp for 5AQ, 665bp for 5BQ and/or 578bp for 5DQ for the bread wheat genotypes. The PCR results revealed that five different alleles were observed from the amplified Q gene loci. The first allele form observed is three different fragments of expected sizes for the Q gene homoeoalleles. This includes 536bp for 5AQ, 665bp for 5BQ and 578bp for 5DQ homoeoalleles. In one third of the tested genotypes (16 genotypes), PCR products for the expected size were observed (Table1).

**Table 1.** Wheat accession demonstrated expected PCR products 5AQ, 5BQ and 5DQ homoeoallele.

SL	Genotype	PCR product of 5AQ (536bp)	PCR product of 5BQ (665bp)	PCR product of 5DQ (578bp)
1	BW1	+	+	+
2	BW4	+	+	+
3	BW120124	+	+	+
4	BW174343	+	+	+
5	BW174444	+	+	+
6	BW174345	+	+	+
7	BW172576	+	+	+
8	Enkoy	+	+	+
9	ETBW9180	+	+	+
10	Bobicho	+	+	+
11	BW120074	+	+	+
12	BW120117	+	+	+
13	BW124421	+	+	+
14	BW172657	+	+	+
15	BW120097	+	+	+
16	BW120076	+	+	+

+, present; -, absent



**Fig. 1.** Gel picture showing amplification of PCR products for 5AQ, 5BQ and 5DQ (3 bands as seen for BW120131) and 5AQ and 5DQ (2 bands as seen for BW3) homoeoalleles of the Q gene for the different wheat genotypes.

The wheat breeding lines showing the expected PCR product sizes for the three Q homoeoalleles includes BW1, BW4, BW120124, BW174343, BW174444, BW174345, BW172576, Enkoy, ETBW9180, Bobicho, BW120074, BW120117, BW124421, BW172657, BW120097, and BW120076 (S Fig1A, S Fig1C, S Fig1D, S Fig1F).

The second allelic form observed was the PCR products of expected size from homoeoalleles of 5AQ and 5DQ, but no 5BQ (Table2). Six genotypes shown PCR amplification of expected product size for 5AQ and 5DQ. These genotypes includes BW174393, BW174395, ETBW8816, Kulkul, ETBW94535, and ETBW9108.

**Table 2.** Wheat genotypes with 5B chromosome substitutions missing the Q gene locus.

SL	Genotype	PCR product of 5AQ (536bp)	PCR product of 5BQ (665bp)	PCR product of 5DQ (578bp)
1	BW174393	+	-	+
2	BW174395	+	-	+
3	ETBW8816	+	-	+
4	Kulkul	+	-	+
5	ETBW 94535	+	-	+
6	ETBW9108	+	-	+

+, present; -, absent

*Detection of length variation in the homoeoalleles*

The experimental result revealed three forms of length variations in the 9<sup>th</sup> intronic region for 5AQ and 5DQ homoeoalleles. The first length variation was the one which depicted for BW2 5AQ with approximate PCR product size of 525bp.

This kind of variation was not detected on other PCR products (Table 3). The second length variation observed from the PCR result was the approximate PCR product sizes of 530 for 5AQ and 585 for 5D Q gene homoeoalleles. This kind of PCR products were observed for the three (3) wheat genotypes such as BW120131, BW174101, and BW174335 (Fig.1; S Fig1A, S Fig1C, S Fig1D, S Fig1F ). The third length variation observed from the PCR result was the approximate PCR product sizes of 540 for 5AQ and 590 for 5D Q gene homoeoalleles.

This kind of PCR products were observed for the seventeen (17) wheat genotypes such as BW3, BW120077, BW172628, BW1727750, BW172750, BW174390, BW172590, Tusie, ETBW9029, BW174364, BW172766, BW174401, BW172576, BW2626, BW174345, BW172683, BW172653, and BW120081 (Table 3). The fourth type of length variation observed is the approximate PCR product sizes of of 550 for 5AQ and 600 for 5D Q gene homoeoalleles. Such heavier PCR products were recorded for two genotypes namely, BW172638, and BW120151. In the above mentioned genotypes showing length variations, there was no amplified PCR product for 5BQ recorded (Table1, Table 2, and Table 3).

**Table 3.** Wheat genotypes with length variations in 5A and 5D Q gene loci.

SL	Genotype	App. PCR product of 5AQ (bp)	App. PCR product of 5BQ (bp)	App. PCR product of 5DQ (bp)
1	BW2	525	-	578
2	BW120131	530	-	585
3	BW174101	530	-	585
4	BW174335	530	-	585
5	BW3	540	-	590
6	BW120077	540	-	590
7	BW172628	540	-	590
8	BW172750	540	-	590
9	BW174390	540	-	590
10	BW172598	540	-	590
11	Tusie	540	-	590
12	ETBW9029	540	-	590
13	BW174364	540	-	590
14	BW172766	540	-	590
15	BW174401	540	-	590
16	BW172576	540	-	590
17	BW172626	540	-	590
18	BW174345	540	-	590
19	BW172683	540	-	590
20	BW172653	540	-	590
21	BW120081	540	-	590
22	BW172638	550	-	600
23	BW120151	550	-	600

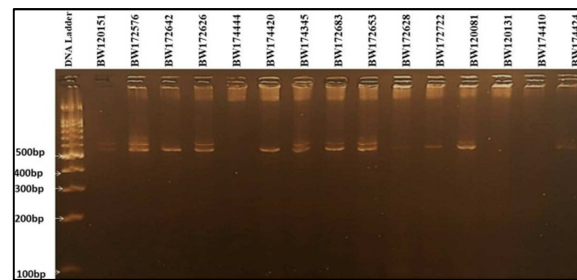
+, present; -, absent

The seventh interesting allelic form was with the approximate PCR product sizes of 536 for 5AQ gene homoeoalleles. PCR products for 5BQ and 5DQ homoeoalleles were not detected for these genotypes. This kind PCR products were observed for the seven (7) wheat genotypes such as BW172621, BW172642, BW174420, BW172722, BW174395, BW174330, and BW172661.

**Table 4.** Wheat genotypes with PCR amplified product for 5AQ, but not for 5BQ&5DQ.

SL	Genotype	PCR product of 5AQ (536bp)	PCR product of 5BQ (665bp)	PCR product of 5DQ (578bp)
1	BW172621	+	-	-
2	BW172642	+	-	-
3	BW174420	+	-	-
4	BW172722	+	-	-
5	BW174395	+	-	-
6	BW174330	+	-	-
7	BW172661	+	-	-

+, present; -, absent



**Fig. 2.** Gel picture showing amplification of PCR products for 5AQ only (single band as send in BW172642) and 5DQ (2 bands as seen for BW172653). The 5BQ and 5DQ bands are missing in some of the wheat genotypes.

**Discussion**

Agriculture will need to produce about 50% more food in 2050 due to a growing world population and changing diets (Ingram, 2017). Wheat, one of the most widely cultivated staple foods in the world, provides 20% of the calories and protein required for human nutrition (Tilman *et al.*, 2011). According to Savary *et al.* (2019), increased wheat production faces biotic (microbial diseases) and abiotic (water, temperature, climate change) challenges. It is estimated that 21.5% of current yield losses are caused by pests and diseases that are part of the biological constraints that threaten global food security. Genetic diversity is therefore important for revealing the presence of changes in genetic makeup, which is crucial for forming the basis for effective selection (Kumar *et al.*, 2013). The Q gene, which is responsible for ideal plant architecture development in wheat, demonstrated to play role in controlling spike length, spike morphology, grain number per spike, and the weight of 1000 grains (Fisher, 2011).

Therefore, influencing grain yield can be achieved by manipulating domestication related traits such as plant height, spike length, spikelet number and spike morphology (Xie *et al.*, 2018; Jee *et al.* 2019). However, further investigation is also needed to identify single nucleotide variations in the 5BQ and 5DQ loci.

DNA marker was used to predict the presence of 5AQ, 5BQ and 5DQ homoeoallele, all of which were detected with various frequencies among the tested entries. When molecular marker applied to detect the 5AQ, 5BQ and 5D Q gene homoeoalleles, indicative expected DNA fragments size of 536bp, 665bp and 578bp, respectively, were observed in 17 bread wheat genotypes BW1, BW4, BW120124, BW174343, BW174444, BW174345, BW172576, Enkoy, ETBW9180, Bobicho, BW120074, BW120117, BW124421, BW172657, BW120097, and BW120076, indicating that the PCR products were from the locus 5A, 5B and 5D. Therefore, the primer can be used for efficiently distinguish Q gene from different homologs of chromosome 5 of hexaploid wheat.

The PCR product size of 665 didn't amplified in thirty (30) wheat genotypes for the 5BQ locus. This indicates that the 5BQ carrying segment of chromosome 5B is substituted with other DNA segment. In wheat genome, 5BQ known to underwent pseudogenization. However, it is transcriptionally active, known to produce truncated protein without the AP2 DNA binding domain and influences the regulation of expression of other homologs (Zhang *et al.*, 2011). Therefore, absence of this locus and its influence in the expression of other homologs needs further investigation.

In twenty three (23) bread wheat accession, intronic length variation in 5AQ and 5DQ were observed. Such variations can be depicted from the PCR product sizes of 525bp, 530bp, and 540bp for 5AQ homoeoalleles and 580bp, 590bp and 600bp for 5DQ homoeoalleles in these genotypes. In addition to length variation in the intronic region of 5AQ and 5DQ, SNP variations were reported from the exonic regions of these gene loci. The SNP variations in the exonic regions of 5AQ and 5DQ

reported to contribute to ideal plant architecture such as plant height; spike length, spike morphology and grain yield (Qiao *et al.*, 2022). However, variations in exonic and intronic regions of the genotypes and their influence on domestication related traits deserve further investigation by DNA sequencing.

The source of A genome for all hexaploid wheat was *T.urartu* and the source for B and G genome was *Ae.speltooides*, whose S genome is the ancestor for B and G genomes in wheat. The source of D genome for hexaploid wheat is *Ae.tauschii* spp.*strangulata*, but not *Ae.tauschii* spp.*tauschii*. Pedigree analysis on the wheat lines showed that, the wheat line such as BW174330 have crossing history with the wild relatives of wheat (*Aegilops squarrosa*), which also give a clue for chromosome substitution in the tested genotypes. PCR band pattern similar to BW174330 have been observed in six more genotypes such as BW172621, BW172642, BW174420, BW172722, BW174395, and BW172661 (Table 4). This suggests that the 5BQ loci may be absent or substituted with other chromosomal parts during the development of the wheat lines.

One of the interesting features of wheat genome is that its intergenic regions of the Q gene loci composed primarily of transposable elements. As a result nested insertion and deletions are common in wheat genome. Most of the variations in the Q gene were reported from exon 1, exon 9, exon 10 and intron 9 (Sormacheva *et al.*, 2015). This study showed thus length (size) variation in the 9<sup>th</sup> intronic region of 5AQ and 5DQ gene loci were observed in twenty three (23) wheat entries examined. Three mechanisms were proposed that might have involved in generation of length and sequence variations and regulation of this gene. First, the intronic regions of Q gene may accumulate active transposable elements which may have impact on gene regulation. Secondly, if the regions contain intronic heterochromatin, epigenetic silencing mechanism might have involved in gene regulation, as seen in rice (Espinosa *et al.*, 2020). Thirdly, this intronic region may be playing role in alternative splicing.

In wheat homologous genes, alternative splicing is known to create partitioned ancestral functions among homoeoalleles (sub functionalization) or in the evolution of novel functions among homoeoalleles (Yu *et al.*, 2020)

### Conclusion

The 9<sup>th</sup> intronic region of the Q gene, which is responsible for domestication related traits such as plant height, days to heading, 1000grain weight, spike morphology and yield have shown variability in Ethiopian wheat genotypes. Presence of genetic variability in crops is essential for its further improvement by providing options for the breeders to develop new varieties and hybrids. Molecular characterization of the Ethiopian bread wheat lines showed the presence of high genetic variability with a large amount of mutations in the intron 9 of the Q gene homoeoalleles. In addition, the genetic materials involved in group 5 homologous chromosomes were identified. The information obtained on the Q gene profile will contribute towards diversification and knowledge based uses of Q gene in Ethiopian wheat breeding programs. This information will be useful in the attempts to develop cultivars with ideal plant architecture and high yield. However, further characterization of the genotypes for domestication traits related phenotypes and the Q gene DNA sequence is necessary.

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### Competing interests

The author declares that there are no conflicts of interest concerning the work described in this manuscript.

### Authors' contributions

SBS and KDD performed the experiment; GHG conceived the project and wrote the manuscript. All authors reviewed the manuscript.

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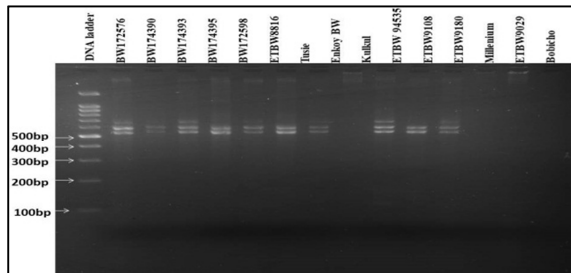


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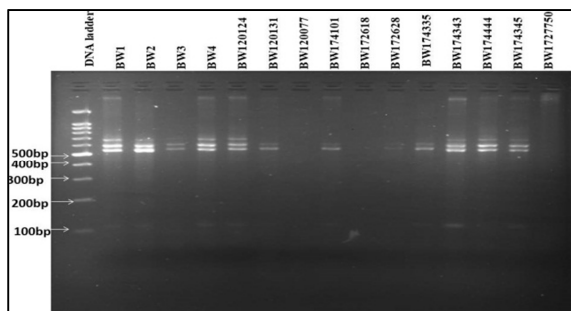
Zhang Z, Belcram H, Magdelenat G, Couloux A, Samain S, Gill S, Rasmussena JB, Barbed V, Faris JD, Huneau C. 2011. Duplication and partitioning in evolution and function of homologous Q loci governing domestication characters in polyploid wheat. *Proceedings of the National Academy of sciences USA* 108, 18737-18742. Doi.org/ 10.1073/pnas.1110552108

**Supplementary materials**

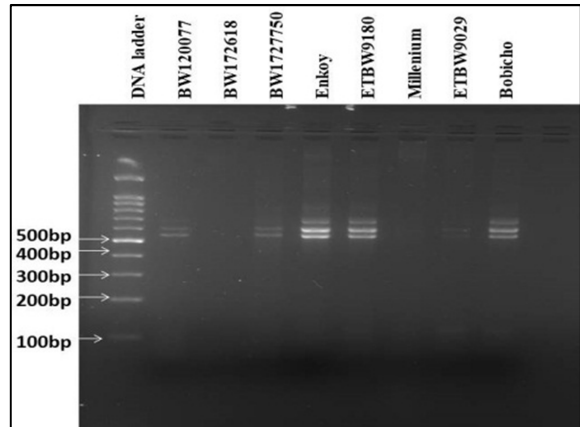
Original and unprocessed gel picture of the tested wheat genotypes for Q gene locus amplification



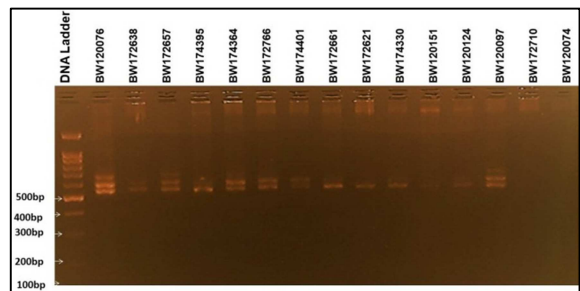
Supplementary Fig. 1A



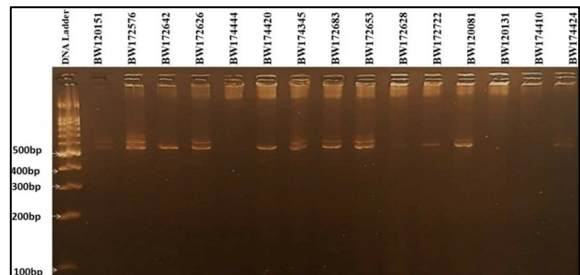
Supplementary Fig.1B



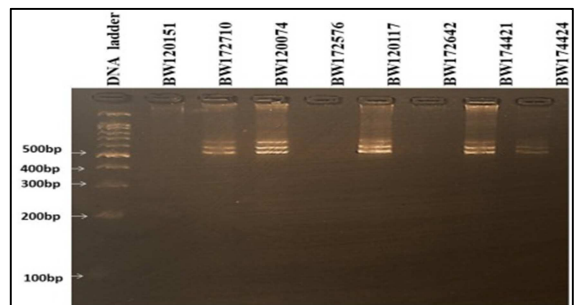
Supplementary Fig. 1C



Supplementary Fig 1D



Supplementary Fig. 1E



Supplementary Fig. 1F