INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 10, p. 66-72, 2013

RESEARCH PAPER OPEN ACCESS

Phylogenetic and *in silico* analysis of *Q* gene in wild and cultivar wheat's

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Key words: *Q* gene, wheat, domestication, phylogentic analysis.

doi: http://dx.doi.org/10.12692/ijb/3.10.66-72 Article published on October 05, 2013

Abstract

Domestication of plant and animals is presses that provide better used of them for human. Wheat is one of the oldest crops that have been domesticated 10,000 years ago. *SOS* and *Q* genes have important role in wheat domesticated. Relationship and phylogenetic of *Q* gene in wheat and wild relatives is very important for breeders. So, new methods such as bioinformatics can be used for this purpose. Bioinformatics analysis display that there is high conservative region among wheat and wild relatives for *Q* gene. Distance-matrix indicated that *Triticum timopheevii* and *Triticum spelta* have highest and lowest similarity with *triticum aestivum* at nucleotide level. Also phylogenetic three using UPGMA method show that *Triticum timopheevii* with *Triticum aestivum* are very closed together that it is possible that *Q* gene from *T. timopheevii* (AA genome) via introgression arrived to *T. aestivum* (AABBDD genome).

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Introduction

Wheat, rice and maize are the three main crops that provide most calories that used by humans. Domestication of plants and animals is process in order to make better use of their cultivation and upbringing by humans (Brown., 2010). It's had been done 10,000 years ago by humans primarily (Feldman *et al.*, 2001). Wheat is one of the oldest crops that has been domesticated and cultivated (Zohary *et al.*, 2000). Bread wheat (*Triticum aestivum*, 2n=6x=42) formed via spontaneous hybridization between tetraploid wheat *T. turdidum L* (2n= 4x= 28, AABB genomes) with diploid *Aegilops Tauschii* Coss(2n=2x= 14, DD genome)(Kihara., 1994). Today, hexaploid bread wheat (*Triticum aesticum*, 2n=6x=42) and tetraploid (2n=4x=28) are new cultivars of wheat that grown widely in the world (Feldman *et al.*, 2001) that 95% of the wheat production in the world belong to bread wheat and the durum wheat other 5%. (1289). Peng (*et al.*, 2003) using F_2 segregation population measured 11 major characteristic that involved in domesticated and identify seven QTL that located on 1BS, 1BL, 3AS, 2AL and 5AL. Three genes *tenacious glumes* (*tg*), *brittle rachis* (*br*) and *Q* genes identify that have important role in wheat domestication. The function of *Q* locus is very complicated that has influences on genetic background. *Q* gene Beside on speltoid and squarenss spike influence on other characters such as heading date, plant height, spike length, spikelet size, seed fertility, glume tenacity, rachis fragility and threshability (Jantasuriyarat *et al.,* 2004; Kato *et al.,* 1999; Kato *et al.,* 2003). It is generally believed that *T. turgidum* as tetraploid progenitor of hexaploid wheat contains *q* allele. It has been debated that as to whether *Q* gene arose one or more than one in tertaploid or hexaploid wheat. *Q* and *q* allele are dosage dependent that maybe *Q* arose from the *q* by duplication that Muramatsu (1963) and Sears (1952 and 1954) show evidence for duplication in dosage response of *Q* gene. *Q* gene involve free-threshing character, square spike phenotype and other characteristic important for domestication such as rachis fragility (Leighty *et al.*, 1921), glume shape and spike length (Muramatsu., 1963). The emergency of the free-threshing with reduce rachis fragility and tenacity gelume allowed farmer to harvested efficiently grain. Also, in the modern agriculture more needed to realized *Q* gene in new cultivars because non-shatering free-threshing grain is very important for mass production. So, *Q* allele have important role in domestication but function, nature and structure of the *Q* allele and relationship with q allele have long been debated.

Economic importance of wheat has been led to cytogenetically and genetically study in the past decades that have resulted in cultivars with high quality and yield and also enhance resistance to biotic and abiotic stress tolerance (Carver, 2009). In contrast, genomics studies in wheat have been delayed due to huge genome sizes (15.961 Mb for bread wheat and 11.660 Mb for durum wheat) and elaboration of genomes (Bennett *et al.*, 2010). Lately, the condition is quite different and convergence of new technology has led to development of robust genomics programs in wheat (Feuillet *et al.*, 2009). Chinese Spring is free-threshing wheat with a square spike and considered to have Q gene. Hope is free-threshing hexaploid wheat considered to posses Q, but it does not have a square spike. Sears (1956) substituted the Hope 5A chromosomes for the Chinese Spring 5A chromosomes in the Chinese Spring background. He observed a square spike indicating that it was the genetic background in combination with *Q* that lent a square spike. Sears and others (Huskins 1946; Muramatsu 1963; Sears 1952; Sears 1954) also developed various aneuploids with varying numbers of chromosome 5A and therefore varying numbers of *Q*. One copy resulted in a speltoid spike, two in a square or normal spike, three in a subcompactoid spike, and four in a compactoid spike (Huskins 1946; Muramatsu 1963; Sears 1952; Sears 1954). This suggested that spike morphology was dependant on the dosage of *Q*. the work Sears and Muramatsu showed that Q is dosage dependent and that *q* is not a deficiency because five copies of *q* resulted in the equivalent of the square spikes observed with two copies of *Q*. Previous research Kunkuck(1959) has indicated that Q evolved as a doubling of q due to unequal crossing over. In study Peleg et al (2011) thresh ability (i.e. the proportion of threshed grain from a spike) was conferred by six QTLs (2B, 4A1, 4A2, 4B, 5A, and 7B), The QTL on chromosome 5A corresponds to the known Q.

In this study, we used bioinformatics methods to illustrated phylogenetic analysis of *Q* gene in wheat and progenitors to identify relationship of them and which wheat is the primarily ancestor of the *Q* gene.

Material and methods

All sequences for Q gene from the different wheat's from the NCBI (National Center for Biotechnology Information) has been downloaded and done nucleotide blast again all wild progenitor of wheat (*Aegilops*) to find similarity sequence (Table 1). In order to alignment of sequences, CLASTALW Analysis of data has been done using Bioedit and Megaline software used for alignment sequence to find similarity and divergence of sequences and also construction of phylogenetic three. Also, *in silico* analysis has been done to find specific restriction enzyme to further research.

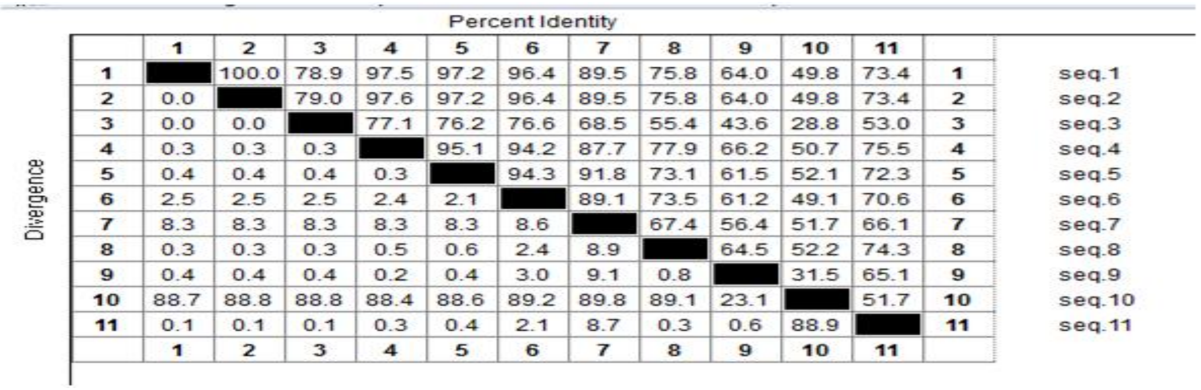
Result and discussion

All sequences from Table.1 showed that there are different among samples based on length of DNA or cDNA. Hence, two samples (*Triticum aestivum* and *Triticum timopheevii*) completely equal in length and among wheat and progenitor of wheat *Triticum turgidum* and *Triticum urartu* have maximum (4958 bp) and minimum (3594 bp) length respectively. So, these results demonstrated that *Triticum turgidum* and *Triticum aestivum* samples with *Q* allele have highest length rather than *Triticum urartu* that has q allele.

Table 1. description of wheat genotypes used for sequence analysis.

Accession number	Length cDNA or DNA	Species	Number sequence
AY702956	3729DNA	*Triticum aestivum*	Seq.1
JX524751	3729 DNA	*Triticum timopheevii*	Seq.2
AY702955	4958 DNA	*Triticum turgidum*	Seq.3
JX524759	3674 DNA	*Triticum dicoccoides*	Seq.4
AY702958	3594 DNA	*Triticum urartu*	Seq.5
JX524744	3769 DNA	*Triticum monococcum*	Seq.6
EU350482	3620 DNA	*Aegilops tauschii*	Seq.7
JX524765	4224 DNA	*Triticum sphaerococcum*	Seq.8
JX524761	4425 DNA	*Triticum spelta*	Seq.9
AY069953	1815 DNA	*Hordeum vulgare*	Seq.10
JX524766	4370	*Triticum flaksbergeri*	Seq.11

Table 2. Similarity and divergence in samples for *Q* gene.



Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11		
1	■	100.0	78.9	97.5	97.2	96.4	89.5	75.8	64.0	49.8	73.4	1	seq.1
2	0.0	■	79.0	97.6	97.2	96.4	89.5	75.8	64.0	49.8	73.4	2	seq.2
3	0.0	0.0	■	77.1	76.2	76.6	68.5	55.4	43.6	28.8	53.0	3	seq.3
4	0.3	0.3	0.3	■	95.1	94.2	87.7	77.9	66.2	50.7	75.5	4	seq.4
5	0.4	0.4	0.4	0.3	■	94.3	91.8	73.1	61.5	52.1	72.3	5	seq.5
6	2.5	2.5	2.5	2.4	2.1	■	89.1	73.5	61.2	49.1	70.6	6	seq.6
7	8.3	8.3	8.3	8.3	8.3	8.6	■	67.4	56.4	51.7	66.1	7	seq.7
8	0.3	0.3	0.3	0.5	0.6	2.4	8.9	■	64.5	52.2	74.3	8	seq.8
9	0.4	0.4	0.4	0.2	0.4	3.0	9.1	0.8	■	31.5	65.1	9	seq.9
10	88.7	88.8	88.8	88.4	88.6	89.2	89.8	89.1	23.1	■	51.7	10	seq.10
11	0.1	0.1	0.1	0.3	0.4	2.1	8.7	0.3	0.6	88.9	■	11	seq.11
	1	2	3	4	5	6	7	8	9	10	11		

Alignment by clustalW method

ClustALW of all sequence display that there are several conservative region in these genes in all samples. Alignment results show that most samples have high similarity except *Aegilops tauschii* that show some insertion and deletion in all part of the gene as well as result illustrated that *Aegilops tauschii* show more variation for these genes. Also, in *Hordeum vulgare* there is a big deletion about 2Kb in the start of the sequence. If the gene divided in two part it can be see that Single Nucleotide Polymorphism (SNP) in the part one more than second part.

Sequence Distance

Distance-matrix used to identify similarity and divergence among samples (Table 2). Table 2 display that *Triticum aestivum* and *Triticum timopheevii* both are quite similar at nucleotide level (100%) and there is not any variation between them. After that, *Triticum dicoccoides* and *Triticum urartu* have show greatest similarity 97.5 and 97.2% respectively. Moreover, *Hordeum vulgare* has only 49.8% similarity with *Triticum aestivum* and *Triticum turgidum* with *Hordeum vulgare* has lowest similarity (28.8%) at nucleotide level. In contrast, *Triticum aestivum with Triticum timopheevii and Triticum turgidum* have not differences at nucleotide level and *Hordeum vulgare* show highest difference with other samples. Also, phylogenetic analysis using UPGMA method illustrated that all samples located in three clusters. *Hordeum vulgare* and *Triticum turgidum* alone has been located in the one cluster and other samples in other clusters. The most wheat samples are located in the cluster that contains two sub-clusters that *Triticum spelta, Triticum sphaerococcum* and *Triticum flaksbergeri* are in a sub-luster and the rest located in the another sub-luster. Based on phylogenetic analysis *Triticum aestivum* and *Triticum timopheevii* are very closed together this means that as previous studies have started that *Q* gene and *SOS* have located on A genome. So, we can concluded that Q gene from A genome via introgression from *Triticum timopheevii* (A genome) transfer to *Triticum aestivum* (AABBDDgenome) (Fig.1)

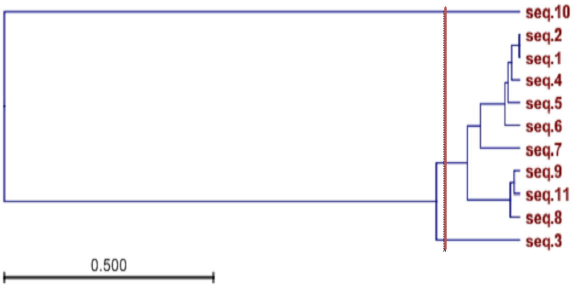


Fig. 1. phylogenetic analysis using UPGMA method using Megalign software.

Insilico analysis

Sequences of two different wheat (seq.1 and seq.5 number) from table.1 that contain *q* and *Q* alleles for *insilico* analysis have been selected. Consensus sequence created from two sequence using Bioedit software then consensus sequence import to NEBcutter V2.0 to find specific enzyme that can be digested one of the sequence and used for distinguish between two alleles. So, result indicated that only *EcoNI* enzyme can be digested sequence from *Triticum aestivum* (*Q* allel) and created two bands 1558 and 2171 bp whereas this enzyme cannot be digested sequence from *q* allele (Fig.2)

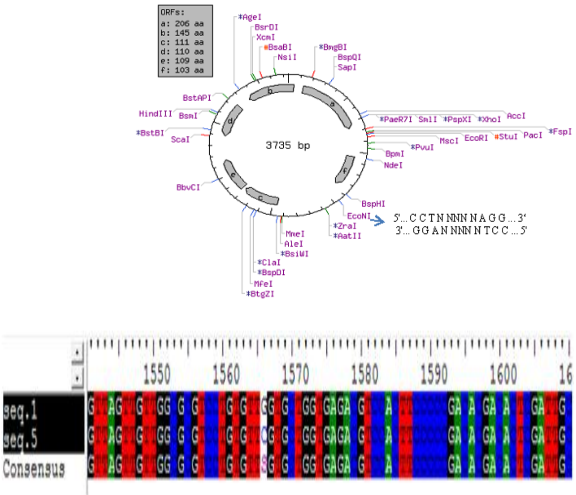


Fig. 2. *in silico* analysis to identify specific restriction enzyme.

Discussion

Free-threshing trait is an important step in the evolution of the wheat under domestication. Wheat's with this property has tinny glume and shell which can provide exit of seeds(peng *et al.*, 2011). *SOS* and *Q* gene are major genes in domestication wheat that has located on A genome. So, A genome rather than B genome has more effect on domestication process in wheat. Gupta (*et al.*, 2008) believed that the diploid wheat genome A carrier is first domesticated wheat. Hence, most traits correlated with domesticated in different wheat has been selected of genome A. in general C, S,k and q factors are known for spike morphology. Factor C from *T.aestivum ssp.compactum* was described that cause compactness of spike and factor K by Watkins (1927; 1928; 1940) is responsible for speltoid spike. S factor create spherical seeds in *T.aestivum ssp.sphaerococcum*. Factor q, initially was considered as series of unknown factors responsible for controlling the morphology of the spike square Philiptschenko (1934). Mackey *et al.*, (1954) showed that the q and k factors exactly the same as in combination with Q factor all tetraploid wheats carrier q allel except *T.turgidium ssp.carthlicum* that have Q allel, free-threshing and circular glume traits(simons., 2005). Nature of Q gene analyzed using deletion and transformation mutants. Accordingly, q allele is basic and only one mutation in q allele rise Q allele and leading to widespread of wheat in the world. But, transcription of Q allele greater than q allele that this led to form square-shaped spike with a good thrashing. All progenitor wheat's with non free-threshing trait have recessive allele and all tetra and hexaploid wheat's with free-threshing have Q allele (peng *et al.*, 2011). Disruption of Q gene caused mutants with q phenotype as spelta. Wheat lines carrying both Q and q alleles represent intermediate phenotypes(peng *et al.,* 2011). It is also TG gene that one of the major genes in domestication processes inhibit expression of Q gene and resulted non free-threshing.TG gene is a semi-dominant gene that has been located on 2D chromosome in *Ae. taushii* so that, hybridization between homozygote tetraploid that have free-thresing trait with Q allele with *Ae. thaushii* created synthetic hexaploid with non-free-threshing that means *tg* inhibited of expression of *Q* allele(villareal *et al.,* 1996). So, hexaploid wheat with free-threshing has tgtg-QQ genotypes(Kerber *et al.*, 1974; Villareal *et al.*, 1996). Primary experiment on cytological analysis of aneuploid show that Q gene is located on 5A chromosome (Huskins., 1946; Unrau *et al.* 1950; Sears., 1952 and 1954; Mackey., 1954). Huskins (1946) and Sears (1952,1954) investigated the Q allele dose effects on morphology of *T.aestivum* and Chinese spring and found that nullisomic, monosomic,disomic, trisomic and tetrasomic for 5A chromosome have spetoid, semispeltoid, square, subcompactoid and compactoid spike respectively. Archeological records indicated that hexaploid and tetraploid wheat with free-threshing trait at the same time about 10000 years ago there have been spelt. This indicated that neither Iranian nor Europeans spelt are capable of free-threshing in hexaploid wheat's (reviewed in Nesbitt and Samuel., 1996; Feldman ., 2001). It is possible that first time Q allele there was in similar tetraploid like *T.turgidium ssp* or extinct T.turgidium and after hybridization with *Ae.taushii* formed first hexaploid with Q allele. Alternatively, Q allele may be existing in hexaploid and tetraploid today got Q allele via secondary hybridization from hexaploid wheat. Anyway, either Q allele mutation that gave rise to the emergence of agriculture and quickly became a prominent factor leading to widespread crop (simons *et al.*, 2006).

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