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Phylogenetic analysis of waxy genes in wheat's using bioinformatics methods

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Abstract

Quality of wheat is depending on three waxy genes. In order to identify variation and phylogenetic analysis of waxy genes in wheat and wild ancestor we used bioinformatics methods to clarify them. Results indicated that there is high variation in nucleotide level among our sequences and *Triticum monococcum* with *Triticum urartu* show 97.3% similarity and *Triticum turgidum* with *Aegilops triuncialis* has only 18.5% similarity. Based on Phylogenetic analysis all samples located in two groups that *Aegilops triuncialis* alone has been located in a group and other samples in another group.

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Introduction

Wheat is one of the most important crops of the world that has a significant role in human nutrition. Nutritional value of wheat grain depends on its chemical composition; especially the biological value of protein and about 60 percent of necessary proteins for human is supplied by wheat. One of the main components of wheat seeds is starch which includes about 65-75 percent of its dry weight (Guzman *et al*., 2012). This macromolecule is made of two polymers of amylase and amylopectin (James *et al*., 2003). Amylose is a linear polymer of glucose units bound together by α (4-1) which includes about 20-30 percent of the total starch, and amylopectin whose structure is similar to that of amylosein which the glucose units are bound to each other through α (4-1) and about 5 percent of glucose isbound by α (6-1) results in creating a branch-structure and includes about 5 percent of the total starch (Habibi *et al*., 2012). Important characteristics of starch such as gelation gelatinization and pasting are usually dependent on the amylose / amylopectin ratio (Zing *et al*., 1997). Recently, wheat lines have been created by changing the ratio of amylose / amylopectin and the lines have been used in breeding programs as well as the development of new wheat lines (Yamari*et al*., 2000;Nakamura *et al*., 1995; Kirbuchi-otobe*et al*., 1997). Amylose is digested more slowly than amylopectin and this kind of digestion is very important for health. Wheat lines containing high amylose are used for producing healthy foods because the amylase is digested more slowly in the small in testing that beneficial effect for human health. (Higgins *et al*., 2004; Behlall and Scholfiled2005; Toooing and Clifton, 2001); and wheat lines containing less or zero amylose are used for improving the quality of noodle (Oda *et al*., 1980). The amylose In the wheat endosperm is usually synthesized in the amylopelast by a protein bound to starch that the granule bound to starch synthesis (GBSS) are known as waxy protein.Omitting or reducing this protein leads to the omission or reduction of amylose (Nakamura *et al*., 1995). It is a key enzyme for amylose synthesis in wheat seeds (Guzman *et al*., 2011). Because we know it as the common wheat or hexaploid wheat with a genome (2n = 6x = 42 AABBDD)*T.aestivum and* three waxy proteins are present which are encoded by three genes named WX-A1,WX-B1,WX-D1 and located on chromosome7DS,4AL,7AS, respectively; each of them has 11 exons and 10 introns (Murai *et al*., 1999). These genes have high molecular weight (Guzman *et al*., 2012) and detecting the allelic diversity is difficult among them. Full waxy wheat (less amylose) 59-60 KD is deficient in all three alleles while partial waxy wheat lacks one or two waxy alleles (Nakamura *et al*.,1993; Miura &Tanii 1994;Yamamori *et al*., 1995). For instance, some types of deficient waxy wheat lacking gene WX-B1 are preferred for noodles (Saito *et al*., 2008). Later on full waxy wheat are produced by combining three alleles void of waxy position. Waxy protein plays a basic role for the quality of flour. The aim of this study is to determine the similarity and relationship of waxy genes sequence in different species of wheat for future breeding program.

Material and methods

Sequences of waxy genes from different organisms (Table 1) were extracted from National Center for Biotechnology Information Database (NCBI) (http://www.ncbi.nlm.nih.gov/). Sequences of all waxy genes had blasted again all wild ancestors of wheat's based on nucleotide blast. Multiple sequence alignment had been done using CLASTALW method using BioEdit software and also similarity matrix, Phylogenetic three UPGMA construction using MegAlign program.

Results

In this study all 19 sequences of waxy genes from different source organisms used for *in silico* analysis. A based on table 1 length of cDNA in 2 species (*Aegilops Tauschii*, *Aegilops umbellulata*) are completely equal (2893bp) and other 2 species (Triticum *turgidum* , *Triticum turgidum*) are completely equal(2781bp) that are belonging to the *Gramineae* family. This characteristic showed that cDNA sequences of waxy genes in these species remain completely conserved during divergence from

their Common ancestor. *In silico* analysis of waxy gene sequences and its comparison with its homoeolgs in the other plant sequences showed a high similarity between plants and Gramineae family,so clusterd groups based on its.

Table 1. Information of waxy genes sequence from different species.

spiceses	Genome	ploidy	cDNA length	Gene Bank Number	Sequence number
Triticum monocuccum	A^mA^m	2x	2834	AF110373	Seq.1
Triticum urartu	A^uA^u	2x	2822	JN857937	Seq.2
Aegilops Speltoides	S^sS^s	2x	2826	AF110374	Seq.3
Aegilops Tauschii	DD	2x	2893	AF110375	Seq.4
Triticum turgidum	AABB	4x	2781	AB029061	Seq.5
Triticum turgidum	AABB	4x	2793	AB029062	Seq.6
Triticum turgidum	AABB	4x	2729	JN935600	Seq.7
Triticum turgidum	AABB	4x	2781	AB029063	Seq.8
Triticum aestivum	AABBDD	6X	2805	AB019622	Seq.9
Triticum aestivum	AABBDD	6x	2695	HQ338720	Seq.10
Aegilops triuncialis	UUCC	4x	1065	AY841017	Seq.11
Aegilops markgrafii	CC	2x	2875	JX679010	Seq.12
Aegilops searsii	SS	2x	2849	JX679012	Seq.13
Aegilops longissima	SS	2x	2861	JX679019	Seq.14
Aegilops sharonensis	SS	2x	2856	JX679017	Seq.15
Aegilops comosa	MM	2x	2886	JX679004	Seq.16
Aegilops umbellulata	UU	2x	2893	JX679006	Seq.17
Aegilops bicornis	S^bS^b	2x	1235	AF079265	Seq.18
Triticum boeoticum	A^uA^u	2x	1234	AF079285	Seq.19

Table. 2. Similarity and divergence in waxy data using Megalign.

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
1		97.3	91.9	86.7	86.2	91.3	84.7	86.4	86.2	83.3	20.6	87.2	90.8	90.4	89.9	88.2	86.8	43.3	46.8	1
2	1.7		90.8	85.8	85.4	90.5	83.9	85.5	86.0	82.4	20.8	87.0	90.8	90.0	89.6	87.8	86.4	43.6	46.9	2
3	6.0	6.4		86.9	86.4	93.1	85.0	86.5	86.4	83.6	20.5	87.2	93.4	91.9	91.3	87.7	87.0	42.9	43.9	3
4	9.0	9.1	9.2		83.4	85.6	82.0	83.6	83.4	80.6	18.6	91.2	87.6	87.5	87.0	92.6	93.6	41.3	40.9	4
5	9.5	9.5	10.3	11.9		86.9	95.8	99.5	98.9	96.4	23.0	84.0	86.0	86.6	86.3	84.2	83.8	42.7	42.9	5
6	6.6	6.7	4.1	8.8	9.9		83.5	87.1	86.2	84.1	21.9	86.2	90.7	89.6	89.3	86.4	85.9	44.4	45.9	6
7	9.3	9.3	10.0	11.6	0.5	9.6		96.1	95.9	98.4	24.7	82.7	84.7	85.0	84.8	82.9	82.5	42.8	43.0	7
8	9.2	9.2	10.1	11.6	0.5	9.6	0.1		98.9	96.7	23.0	84.2	86.2	86.8	86.5	84.5	84.0	42.7	42.9	8
9	9.3	9.3	10.2	11.7	0.3	9.8	0.3	0.4		95.7	22.2	84.6	86.6	87.0	86.8	84.8	84.5	41.9	42.1	9
10	9.4	9.5	10.1	11.8	0.5	9.7	0.1	0.1	0.3		25.8	81.2	83.3	83.7	83.4	81.5	81.1	43.9	44.1	10
11	102.3	103.5	103.5	105.1	107.6	107.1	107.5	107.5	107.1	107.0		19.4	19.9	20.4	20.5	19.4	18.7	71.9	71.3	11
12	8.3	8.4	8.8	5.5	10.9	8.3	10.6	10.6	10.7	10.8	98.8		87.7	88.1	87.6	91.8	91.9	41.2	41.5	12
13	6.1	6.1	4.6	8.5	9.7	5.4	9.3	9.4	9.5	9.4	103.3	8.8		90.3	90.3	89.1	88.2	42.3	43.2	13
14	6.8	6.9	6.8	8.5	10.4	7.0	10.4	10.1	10.4	10.3	103.4	8.1	7.7		98.3	89.5	87.5	45.9	42.8	14
15	7.0	7.0	7.0	8.7	10.5	6.9	10.5	10.3	10.4	10.5	103.6	8.3	7.4	1.1		89.0	87.2	46.0	42.9	15
16	7.6	7.7	8.5	5.1	11.6	8.4	11.2	11.2	11.4	11.4	102.7	4.8	7.9	7.6	7.7		93.2	42.8	42.6	16
17	8.3	8.3	8.4	4.8	11.4	8.1	11.0	11.1	11.2	11.2	101.8	5.3	8.1	8.8	8.8	4.9		40.5	40.1	17
18	6.6	6.4	7.6	6.9	12.4	8.6	12.6	12.3	12.4	12.6	104.3	7.2	7.6	0.6	0.7	4.7	7.2		96.5	18
19	0.4	0.8	5.2	7.4	10.0	5.8	10.0	9.9	10.0	10.2	103.3	6.1	4.3	5.9	6.0	3.8	6.8	6.2		19
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	

Divergence

seq.1 seq.2 seq.3 seq.4 seq.5 seq.6 seq.7 seq.8 seq.9 seq.10 seq.11 seq.12 seq.13 seq.14 seq.15 seq.16 seq.17 seq.18 seq.19

Alignment by clustalW method

Multiple sequence alignment of waxy gene sequence showed maximum homology in different regions (Fig 1).Result of cDNA sequence alignment showed for this gene that some sequence have a high similarity and only in small region have a point different that May be due to environmental factors such as mutation. Clustalw alignment indicated that Triticum turgidum and Triticum aestivum have a same insertion in 1846-1855bp and also there is some conservative region in this two species. Also, alignment of all sequences show that there are six conservative region in all

sequences from 257-340, 483-547, 588-606, 715-751, 858-894, 1434-1608 except for 11,18 and 19 number sequences.(Fig 2) Sequence alignment is the pre-requisite for carrying the phylogenetic analysis as well as predicting the second and third structures.

Sequence distance

Distance-matrix methods of phylogenetic analysis main rely On genetic distance between all the sequences at Table 2. Calculate a measure of the distance between each pair of sequences used to identify spices that show high similarity and also divergence. Table 2 show that *Triticum monocuccum* with *Triticum urartu* have 97.3 % similarity in nucleotide level and there is not any polymorphism between these two diploid wheat's (Rodrigueze Quijano *et al* 2004). Also, diploid spices show less variation among them for waxy genes that previous study reported same results (Liu *et al* (2009) and Gazman *et al* (2012). Also, Table 2 show that among wheats, *Triticum turgidum* with *Triticum aestivum* have high similarity (98.9) and *Aegilops longissima* with *Aegilops sharonensis* show 98.3% similarity and lowest similarity (18.6) was for *Aegilops triuncialis* with *Triticum turgidum*. Based on Table 2 Triticum turgidum(AB029063)with Triticum turgidum(JN935600) and *Aegilops triuncialis* (AY841017) with *Triticum turgidum* (AB029063) show lowest (0.1%) and highest (107.5%) differences respectively.

Phylogenetic Tree

Using phylogenetic tree we can identify evolutionary relationships of spices at molecular level that length of each branch show degree of evolutionary divergence. Result of Phylogenetic Tree (Fig 3) showed that species classified in two groups , group 1 consist of 16 plants (*Triticum turgidum, Triticum aestivum, Triticum turgidum, Triticum aestivum, Aegilops Speltoides, Aegilops searsii, Triticum monocuccum, Triticum boeoticum, Triticum urartu, Aegilops longissima, Aegilops bicornis, Aegilops sharonensis, Aegilops Tauschii, Aegilops umbellulata*), group 2 consist of only two plants (*Aegilops triuncialis* and *Aegilops markgrafii*). In the group 1 there are five subclusters that *Triticum aestivum* with *Triticum turgidum* located in the same subcluster that these indicated that maybe during the evaluation of waxy genes via introgression transferred to *Triticum aestivum*. Phylogenies were constructed with waxy gene sequences from all species cleared the evolutionary relationships between these genes. The waxy sequence from (*Aegilops triuncialis*) was assigned into the most distal clade.

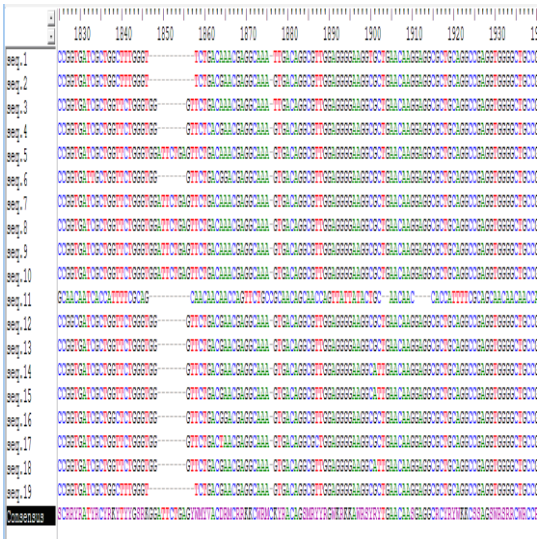

Fig. 1. Multiple sequence alignment cDNA in the part of waxy genes in all sequences.

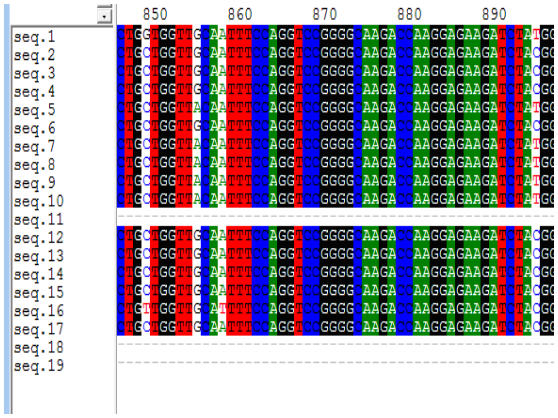

Fig. 2. conservative 854-894 region in all sequence.

Discussion

Wheat is one of the stable crops for thousands of years and have more effective on economic worldwide. Many traits are importance for wheat production that one of them is quality. Quality in wheat is correlated with of waxy genes also as waxy proteins. Waxy proteins increased starch quality in wheat. Three waxy genes (W-A, W-B, W-D) have been identified in the bread wheat. Bread wheat (Triticum *aestivum)* (2n = 6x = 42, AABBDD) is hexaploid

wheat that has formed via spontaneous hybridization and successive chromosome doubling. The first step involved the spontaneous hybridization between *Triticum urartu Thum. Ex Gandil.* (2n = 2x = 14 A^uA^u the wild diploid wheat) and an unknown species (BB) related to *Aegilops speltoides* ((2n = 2x = 14 SS). The resulting tetraploid wheat *Triticum turgidum ssp.durum*(2n = 4x =28 AABB) then hybridized with *Aegilops tauschii Coss* (the diploid ancestor of the D genome, 2n = 2x = DD) to produce hexaploid bread wheat. wild diploid wheat (A genome) is origin for the all polyploidy wheat's.(Dvorak *et al.*, 1988).The variation sequence of waxy gene in the *Aegilops tauschii* is an important source in improving starch quality and also on the polygene of waxy gene in different species that provide useful information (Li *et al.*, 2013).

There are several methods to screening of germplasm for quality trait such as Light measurement method, SDS-PAGE and sequencing for waxy genes. Yamari and *et al* (1994, 1995) identified polymorphism for waxy proteins such as Gluten and Gliding at quantitative level. Recently, Guzman *et al* (2012) used sequencing method to identify phylogenetic relationships among wheat spices for waxy genes and concluded that there are high variations among them also Hang and Bilo (2012) were obtained similar results that our results indicated that there are variation for waxy genes in wheat's and wild ancestors of wheat for waxy gene. Our results also indicated that *Triticum Turgidum* is much closed for waxy genes with *Triticum aestivum* that researcher can used *Triticum turgidum* in quality wheat breeding program. Also wild ancestor show variation for waxy genes and have some insertion or deletion in waxy genes, so, we suggested used wild ancestors in breeding modern wheat.

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