Investigation of diversity of Waxy-A1 gene using amplification in different spices in A genome wheat’s

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ABSTRACT

Granule Bound Starch Syntheses are enzymes that have main role in starch quality. Diploid spices of wheat that contain A genome are the donor of A genome to polyploid wheat. So, in order to identification of variation for waxy gene used some species that involved A genome. Results show that triticum urartu is an ancestor of A genome to polyploid wheat’s show different size for this gene than triticum aestivum. So, bioinformatics’ and sequencing method are necessary to investigate of this variation.

INTRODUCTION

Granule Bound Starch Syntheses (waxy proteins) are the only enzyme that has a role in amylose synthesis in wheat [1, 2]. Three waxy proteins are present in hexaploid wheat (Triticum aestivum L. ssp. aestivum) with molecular weights around 59 - 60 kDa and are encoded by three genes that Wx-A1 located on chromosome 7AS, Wx-B1 on chromosome 4AL, and Wx-D1 on chromosome 7DS respectively [3, 4]. Each gene has eleven exons and ten introns [5, 6]. Several studies have shown that variation in amylose content is associated with the status of expression [7-11] of the three Wx genes. In the major species of diploid wheat (2n = 2x = 14) are T. urartu Thum. ex Gandil and Triticum monococcum L. ssp. monococcum, T. monococcum ssp. aegilopoides Link em. Thell. (syn. T. boeoticum Boiss.). All these species contain the AA genome, which is closely related to the A-genome donor to polyploid wheat’s (durum and bread wheat). Wild diploid wheat usually distributed in the Fertile Crescent region [12, 13].

In diploid wheats, where one exclusive waxy protein is detected (Wx-A1), this protein is encoded by the Wx-A1 gene located on the short arm of the chromosome 7A. Numerous studies have suggested that this species generated wild emmer wheat (T. turgidum ssp. dicocoides Ko’rn. ex Asch. et Graebner em. Thell.; 2n = 28 = 28, AuAuBB) by a spontaneous cross with an Aegilops species (probably Aegilops speltoides Tausch.; 2n = 28 = 14, SS) with later chromosomal duplication. The domestication of wild emmer wheat produced cultivated emmer wheat (T. turgidum ssp. dicoccon (Schrank) Thell.; 2n = 4x = 28, AuAuBB), the predecessor to durum wheat (T. turgidumssp. Durum (Desf.) Husnot; 2n = 4x= 28, AuAuBB) Consequently the A genome of the all polyploid wheats has its origin in this wild diploid wheat [14].

The ancient wheats, along with their relatives, have showed to be an important source of variability for the bread quality and could helpful in the breeding of the modern wheat [15]. Waxy gene from (T. monococcum L. ssp. monococcum; 2n = 2x = 14, AmAm) have been well characterized at the molecular level [16, 17]three partial sequences of T. urartu Wx gene have been reported [18-20]. Also yamamori and Guzman [21] reported five wheat Wx alleles (Wx-A1c,-A1d,-A1e, -Ali and Alj) that produce polymorphic Wx proteins. Also Guzman et al. [22-24]detected different null alleles, including Wx-A1. However, other types of the null Wx-A1 allele have been also identified, for example Saito et al. [25] detected an insertion of 173 bp inside the fourth exon of Wx-A1f that changes the ORF and leads to a premature stop codon. Yamamori et al. [26] found that the null Wx-A1 allele frequently occurred in Japanese, Korean and Turkish cultivars.Saito and Nakamura [27] found an insertion and a deletion of one nucleotide that changed the ORF of the gene in wild and cultivated emmer (T. turgidum ssp. dicocoides Ko’rn. ex Asch. and Graebner em. Thell. And T. turgidum ssp. dicoccon Schrank, respectively) that lacked the Wx-A1 protein.
The aim of this study is to determine the similarity and relationship of wx-A1 gene in the ancient wheats and its comparison with those present in bread wheat together with the analysis of the relationships among different species.

MATERIALS AND METHODS

In this study eleven samples from three species belong to triticum genus has been collected from the West of Iran (in table 1). 10 Seeds of each samples planted in pots with 10 diameters and leaf sample from 4-6 leaves stage have been collected and immediately storage in -20 C for more study.

DNA Extraction and PCR Amplification:

For DNA extraction, about 100 mg of young leaf tissue were excised and immediately frozen in liquid nitrogen. DNA was isolated according to Doelly et al. [28] with some modifications. The primers that have been designed by Shariflou et al [26] were used to amplify the some regions of the Wx-A gene and Wx-D. Sequence of Wx-A1 was Wx-A1R (5-CTTCCCTGAAGAGGAAGAAAGAA-3) and Wx-A F (5-ATAGGGCAACCCCTAC-3). PCR reactions were performed in a total volume of 25μL containing 50 ng genomic DNA (3 μl), 1.5μM MgCl2 , 2.5 μL 10x PCR Buffer, 1.5 μL 1m M d NTPs , 10 pmol of each primer(1μL) ,14.2 μL ddH2O and0.3 Taq DNA Polymerase. The PCR cycle consisted of an initial 4-min denaturation at 94°, followed by 35 cycle of 94° for 45 sec, 54° for 30 sec, 72° for 2 min, and 1 cycle of 72°.A 5 μl aliquot of the PCR mixture were resolved in 1.5% agarose gels, and bands were visualized by ethidium bromide staining.

RESULTS AND DISCUSSION

Results:

The PCR amplification of the Wx-A 1 gene was carried out in genomics DNA with the specific primers designed by Shariflou et al for all samples. [26]. This permitted the simultaneous amplification of the two waxy genes (A, and D). Results from the amplification of this region for Wx-A1 gene in three wild Triticum species only produced one fragment [Fig 1]. Also, no differences were observed among this amplicons for the species that contain Wx-A1a (230bp) and Wx-A1b (265bp) alleles. But, amplification of the region of Wx-A1 gene revealed a remarkable difference between other species with common wheathat used as standard. The size of the Wx-A1a ranged less from 230bp found in Triticum aestivum, which show small deletion for this region. T. boeoticum and T. dicoccoides show similar size (230bp) together, but in T. dicoccoides (sample 11) show small deletion for this region. T. urartu species show different size and more than 265 bp for this primer.

Discussion:

Wild wheat’s are neglected crops that could be used in the quality breeding of modern common and durum wheat. One important aspect of this quality is the starch property which is related to the waxy protein or GBSSI (granule-bound starch synthase), that is the enzyme key for the synthesis of amylose in wheat seeds. Several studies have shown variation in amylase content that is correlated with expression of Waxy proteins [4, 5, 27], two different alleles were found, namely Wx-Am1a and Wx-Am1a. The allele Wx-Am1a was shown to be less mobility than the Wx-Am1a allele that has been proposed as the donor of the A genome in the polyploid species of wheat’s [11]. In this study three wild Triticum species that were analyzed for Wx-A1a/b allele show differences in size (230bp-265bp). These results are being similar to those found by Long-Dou et al [31] but, is not agree with the theory that suggests A genome of the polyploid wheat’s is derived from T. urartu einkorn wheat [32] and also don’t agree with the results of Guzman et al. [10], samalanni et al [32], and Dvorak et al. [14]. Phylogenetic analysis of waxy genes in wheat’s using bioinformatics methods by Maryami et al. [33] show that waxy gene in Triticum urartu (2822 bp) is bigger than Triticum aestivum (2805 bp), so, for more validation of this results is necessary bioinformatics analysis of this region in above samples.

Conclusions:

Exploiting the variation of waxy –A1 in new genetic resources is necessary in the ancient wheat’s and its comparison with those present in bread wheat for analysis of the relationships among different species. Also, allelic diversity of the Wx-A1 within the ancient wheat’s which could be used for evaluating the phylogenetic relationships between different wheat species. In this study different samples show little variation based on size amplification in Wx-A1 except in T. dicoccoides (Sample 11) that there is small deletion for this region. But, Triticum aestivum (sample1) and T. urartu show big deletion than sample 11,so, for more information suggested used sequencing methods for this region and also complete Wx-A1gene amplification in order to identify
diversity in Wx-A1 gene. Also, using sequencing methods we can able be identify more deletion and insertion at Single Nucleotide Polymorphism (SNP) level in *T. boeoticum* and *T. dicoccoides* that maybe help breeder in quality breeding programs.

**Table 1:** Sample of the Wild wheat’s species used in the study.

<table>
<thead>
<tr>
<th>Number</th>
<th>Species</th>
<th>Genome</th>
<th>Ploidy</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Triticum aestivum</em></td>
<td>AABBDD</td>
<td>6X</td>
<td>Iran-Ilam</td>
</tr>
<tr>
<td>2</td>
<td><em>Triticum boeoticum</em></td>
<td>AmAm</td>
<td>2X</td>
<td>Iran-Ilam</td>
</tr>
<tr>
<td>3</td>
<td><em>Triticum boeoticum</em></td>
<td>AmAm</td>
<td>2X</td>
<td>Iran-Abadan</td>
</tr>
<tr>
<td>4</td>
<td><em>Triticum urartu</em></td>
<td>AuAu</td>
<td>2X</td>
<td>Iran-Ilam</td>
</tr>
<tr>
<td>5</td>
<td><em>Triticum urartu</em></td>
<td>AuAu</td>
<td>2X</td>
<td>Iran</td>
</tr>
<tr>
<td>6</td>
<td><em>Triticum urartu</em></td>
<td>AuAu</td>
<td>2X</td>
<td>Iran</td>
</tr>
<tr>
<td>7</td>
<td><em>Triticum urartu</em></td>
<td>AuAu</td>
<td>2X</td>
<td>Iran</td>
</tr>
<tr>
<td>8</td>
<td><em>T. dicoccoides</em></td>
<td>AuAuBB</td>
<td>4X</td>
<td>Iran</td>
</tr>
<tr>
<td>9</td>
<td><em>T. dicoccoides</em></td>
<td>AuAuBB</td>
<td>4X</td>
<td>Iran</td>
</tr>
<tr>
<td>10</td>
<td><em>T. dicoccoides</em></td>
<td>AuAuBB</td>
<td>4X</td>
<td>Iran-Kermanshah</td>
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<tr>
<td>11</td>
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<td>4X</td>
<td>Iran</td>
</tr>
<tr>
<td>12</td>
<td><em>T. dicoccoides</em></td>
<td>AuAuBB</td>
<td>4X</td>
<td>Iran</td>
</tr>
</tbody>
</table>

Fig. 1: Amplification products of the WxA1a/b gene in wild *Triticum* species.

**REFERENCE**


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