ABSTRACT: To identify the high molecular weight Glutenin subunits of 57 wheat landraces of Northwest of Iran by SDS-PAGE an experiment was conducted at Islamic Azad University, Ardabil Branch in 2013. Considerable diversity with respect to three Glu-A1, Glu-B1 and Glu-D1 loci were observed. In Glu-A1 locus 1, 2* and null alleles were found with the frequency of 10.5, 57.9 and 31.6 percent. In Glu-B1, 7 alleles were detected, in which 7+8 allele was the most frequent allele. In Glu-D1 locus, 2+12 and 5+10 were recognized with the frequency of 68.4 and 31.6 percent. The high quality 5+10 subunit pair was recognized in 9 landraces. Quality score of landraces were ranked from 4 to 10 indicating vast genetic diversity among the landraces. Landraces number 1, 20, 23, 27, 51, 52, 36 and 49 had the best composition of HMW glutenin subunits and consequently highest quality score.

Keywords: Electrophoresis, Landrace, SDS-PAGE, wheat

INTRODUCTION

Bread-making quality of wheat is mainly determined by the composition and quantity of gluten-forming storage proteins, in particular the high-molecular-weight glutenin subunits (HMW-GS) (Butow et al. 2003). HMW-GS are significant in determination of the wheat grain processing quality due to their compositional effects on the elasticity of wheat dough. Although HMW-GS constitute only 10% of storage proteins of the wheat endosperm (Payne et al. 1981), they have proved to be of importance in determining genotypic variation in bread making quality of bread wheat from many countries (De Bustos et al. 2001; Rodriguez-QuiJano et al. 2001). Based on the different mobility, revealed by SDS-PAGE, a large number of HMW-GS allelic variations of Glu-1 loci are identified and listed in wheat gene catalogs. The HMW-GS are encoded by genes at three Glu-1 loci: Glu-A1, Glu-B1 and Glu-D1, located on the long arms of homologous group-1 chromosomes (Payne and Lawrence 1983). Each locus is made of two tightly linked genes, e.g., Glu-D1-1 and Glu-D1-2, which encode the x and y subunits, respectively. Among allelic HMW subunits controlled by the Glu-A1 locus on chromosome 1A, bands 1 and 2 have an equal positive effect over the null allele, suggesting a quantitative effect. Similarly, among several alleles at the Glu-B1 locus on chromosome 1B, those producing double bands or intensely staining bands (for example subunits 7+8, 13+16, and 17+18) are associated with superior bread-making quality compared with those with single or faint bands (for example subunits 7, 20, and 6+8) (Singh et al. 1990). Considerable progress has been achieved in the characterization of HMW-GS alleles from wheat and its related species (Jiang et al. 2005, 2009; Shewry et al. 2006). Local varieties of crops and their landraces possess the most suitable genes due to their history and adaptability to biotic conditions and unfavorable environmental factors of the corresponding regions, so they can provide the plant breeders with desired genetic variations (Taeb 1996). Local germplasm, being very valuable sources for breeding programs (Bardsley and Tornas 2005). Since the small part of the Iranian wheat collection has been evaluated and evaluation parameters are mostly agronomic readings, evaluation of biochemical
characters such as those related to baking quality of wheat can have a great importance. The aim of this experiment was the characterization of Iranian bread wheat germplasm for HMW-GS.

MATERIALS AND METHODS

57 bread wheat accessions of Iran (belonged to Ardabil, East and West Azerbaijan and Zanjan provinces) were received from national plant gene bank of Iran and their seeds used for SDS-PAGE in 2012. SDS-PAGE of HMW-GS was carried out in a discontinuous buffer system following the method of Laemmli (1970). For extraction of protein, a single seed was ground to fine powder with mortar and pestle. Four hundred μl of extraction buffer (0.5 M Tris-HCl (pH 6.8), 2.5% SDS, 10% glycerol and 5% 2-mercaptoethanol) was added to a 0.01 g (10 mg) seed powder and mixed thoroughly by vortex in an Eppendorf tube (1.5 ml) with a small glass rod. The mix centrifuged at 15000 rpm for 10 min. Ten μl of sample was loaded in each well. Seed protein was analyzed through slab-type SDS-PAGE using 10% polyacrylamide gel. 7.5% gels also used for separation of subunit. The composition of HMW glutenin subunits encoded at the Glu-A1, Glu-B1 and Glu-D1 loci, determined according to Payne and Lawrence (1983).

RESULTS

In landraces being studied between 3 through 5 subunits were observed in 3 genomes. Twelve allelic variants (3 alleles at Glu-A1 locus, 7 at Glu-B1 locus and 2 at Glu-D1) were found between the evaluated lines (Table 1). At Glu-A1 locus, 1, 2* and null alleles were identified with the frequency of 10.5%, 57.9%, and 31.6%. Abundance of 2* allele at Glu-A1 locus, with positive effect on quality, has developed the quality score of the most of landraces. At locus Glu-B1, 7 subunits namely 6+8, 7+8, 7+9, 7, 13+16, 17+18 and 20 were identified with the frequency of 5.3%, 64.9%, 8.8%, 5.3%, 5.3%, 8.8% and 1.8% respectively. Among 7 alleles of locus Glu-B1, subunit 7+8 and 13+16 are the best alleles. At locus Glu-D1, two subunit pairs namely 2+12 and 5+10 were observed with the abundance of 68.4% and 31.6%, respectively. At locus Glu-D1, 2+12 subunit pair was more frequent than subunit pair of 5+10.

Table 1. Allele frequencies at Glu-1 loci in 57 Iranian landraces of bread wheat

<table>
<thead>
<tr>
<th>Gene loci</th>
<th>Allele</th>
<th>Number</th>
<th>Frequency (%)</th>
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</thead>
<tbody>
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<td>31.6</td>
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<td></td>
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<td>10.5</td>
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<td>2</td>
<td>33</td>
<td>57.9</td>
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<tr>
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<td>5.3</td>
</tr>
<tr>
<td></td>
<td>7</td>
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<td>13+16</td>
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<tr>
<td></td>
<td>5+12</td>
<td>18</td>
<td>31.6</td>
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</table>

HMW-GS composition and quality score of wheat landraces are shown in Table 2. As shown in table 2, landraces number 1, 20, 23, 27, 51, 52 (with 2*, 7+8 and 5+10 composition), landrace number 36 (with 1, 7+8 and 5+10 composition) and landrace 49 (with 2*, 13+16 and 5+10 composition) had the highest quality score. It has been reported that genes coding for D genome subunits play important role in determining the bread-making quality (Rodriguez-Quijano et al. 2001), and good bread quality is mostly associated with the presence of subunit 5+10 at locus Glu-D1 (Bushuk 1998; Rabinovich 1998). 19 different allelic compositions of HMW-GS were observed at locus Glu-1 for 57 lines. Among studied landraces, the most common composition was (2*, 7+8 and 2+12).
Table 2. HMW Glutenin subunits and quality score in genotypes under study

<table>
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<tr>
<th>Row</th>
<th>Line</th>
<th>Glu-A1</th>
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<th>Glu-D1</th>
<th>Score</th>
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</table>
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