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Full Length Research Paper

Designing polymorphic ISSR primers in order to study x and y types glutenin subunits in 1D locus controlling favorable baking quality of bread wheat

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Baking quality is one of most important traits in qualitative improvement of bread wheat. Gluten prolamins determine wheat flour quality for different technological processes such as bread making. Among the gluten proteins, High Molecular Glutenin (HMW) group and specially, x-type and y-type subunits of d allele in 1D locus are very valuable in baking quality. In this study, amino acid sequences of x-type subunits [2.1, 2.2, 2.2*, 5] and y-type subunits [10, 12] related to 1D locus were searched and compared together using Genedoc software. After alignment of amino acid sequences of y-type subunits and x-type subunits, it was characterized that deletion, insertion (duplication) and point mutations in these subunits are involved in biological function of proteins. Finally, polymorph ISSR primers in repetitive domains were designed on similarities and differences in x and y types subunits. After performing PCR and DPAGE, it was found that these primers show good banding polymorphisms in elite mutant lines, standard commercial cultivars and F_2 populations from crosses and are ideal for DNA polymorphisms detection in glutenin subunits of 1D locus.

Key words: Alignment, DPAGE, glutenin subunits, ISSR, baking quality and wheat.

INTRODUCTION

Baking quality is one of important traits in qualitative improvement of bread wheat(3). Proteins of wheat seeds are classified into four groups Albumins, Glubulins, Gliadins and Glutenins. Gliadins and Glutenins contain about 80 (30+50) percent of endosperm proteins. These two storage groups belong to larger group referred to Prolamin. Gluten prolamins determine wheat flour quality for various technological processes such as bread-making. Baking value depends directly on gluten strength and this trait is related to kind of protein. Seed protein content hasn't effect on baking value. Wheat gluten is combination of two physical qualities: elasticity and vescusity that related to polymeric glutenins and monomeric gliadins respectively. Gluten proteins consist of two distinct groups (High Molecular Weight Glutenin and Low Molecular Weight Glutenin). Loci controling High Molecular Weight Glutenins are located on long arm of chromosomes 1A, 1B , 1D and totally, are called GLU-1(3). They are classified into two types on the basis of their M_r values and sequeces: x-types which migrate more slowly on SDS/PAGE and y-types which migrate faster. Amino acid compositions of these subunits contain high contents glycine (14 - 19 mol%), glutamine (37 - 39 mol%) and proline (12 - 14 mol%). High Molecular Weight subunits account for about 10% of glutenin. Structure prediction of glutenin subunits specially High Molecular Weight indicates that the N- and C-terminal domains are predominantly α-helical, while the central repititive domains form regularly repeated β-turns. Turns are formed both within repeat motifs and spanning the junctions between them. It appears that the repetitive domains of the HMW subunits form a novel supersecondary structure based on repeated β-turns. In the HMW subunits, the cross-linking sites (cysteine residues) are predominantly in the N- and C-termina domains, which would allow the formation of head-to-tail polymers with some branching and cross-linking. Some of the cystein residues may also form intrachain disulphide bonds, at least in the y-type subunits. The precise number and distribution of the

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Table 1. Names of mutant genotypes and parents and commercial cultivars.

Tabasi	T-58-8	O-6-1-1	Ro-9	Navid
T-64-9-Ip	T-58-7	Roshan	Ro-10	Karaj-1
T-66-58-6	T-66-I-II	Ro-1	Ro-11	Tajan-garm
T-66-58-9	T-67-60	Ro-2	Ro-12	As-48
T-65-6	T-65-9-II-4	Ro-3	Azadi	Azar-mutant
T-65-5-1	T-66-58-60	Ro-4	Azar	Inia*
T-66-58-10	T-58-14	Ro-5	Tajan	Navid
T-66-58-12	Omid	Ro-6	Bezostaya	Atrak
T-65-9-1	O-64-1-1	Ro-7	Pishtaz	
T-67-7-1	O-64-4	Ro-8	Chinese spring	

*Unfortunately, Inia cultivar has favorable 5+10 allele naturally but, becuase it was heterozygote (not elite), not was detected favorable 5+10 allele in gel profile.

cross-links influenced the elastic modulus of gluten, and differences of this type could be responsible for allelic variation in breadmaking quality. Considering that glutenin subunits specially High Molecular Weight are rich in glutamine and glycine, deletion/insertion of these sequences rich has imporatant role in their biological function. In addition to these deletion, insertion and substitution mutations, functional and expressional point mutations are detectable in study of glutenin subunits sequences. Among these mutations, it can be pointed to substitution or conversion of amino acids involved in secondary and tritiary structures of proteins (α-helix and β-sheet). Among gluten proteins, High Molecular Glutenin (HMW) group and specially, x-type and y-type subunits of d allele in 1D locus are very valuable in baking quality. As x-type and y-type subunits of glutenin genes are rich in repeat motives, designing of polymorphic ISSR primers can be very profitable for evaluation of glutenin genes in commercial cultivars and favorable mutant lines after irradiation and in order to identify polymorphic markers linked to target trait.

MATERIALS AND METHODS

In this study, it was used from several favorable mutant lines available in Nuclear Agriculture Department and some commercial cultivars of wheat as standard. Some commercial cultivars such as Attrak have favorable 5 + 10 allele but others such as Tabasi have no this allele (Table 1).

Also, in this research, amino acid sequences of x-type subunits [2.1, 2.2, 2.2*, 5] (GenBank accession nos. AY517724, AY159367, AJ893508, and X12928)(6,7,8,4) and y-type subunits [10, 12] (GenBank accession nos. X12929 and AY486484)(4,5) related to 1D locus were searched in NCBI site and compared together or independently using Genedoc software. Then, nucleotide sequences of similar domains due to alignment of amino acid sequences are searched, compared and aligned using this software. Several ISSR primers were designed on similarities and differences in repetitive motives of x and y types subunits in 1D locus.

After DNA extraction by CTAB method, amplification operation was done in thermocycler by PCR and using ISSR primers and banding patterns were detected on polyacrylamide gel.

Exprimental Results

After alignment, it was observed similarities and differences in repetitive motives of x and y types subunits in 1D locus (Figure 1). Among ISSR designed primers, six primers were selected for polymorphism study of repetitive motives (Tables 2 and 3).

DNA extraction was done and DNA samples were prepared from elite mutant lines, standard commercial cultivars and F_2 generation plants from crosses between two mutant lines and two commercial cultivars. After determination of DNA quality and concentration, PCR was carried out in a thermal cycler with a 96 tube holder block. PCR operation consisted of initial denaturation at 96°C for 7 m, 30 cycles of denaturation at 96°C for 30s, anneling at 54 - 60°C for 45s and extension at 72°C for 2 m, followed by a final cycle of extension at 72°C for 7 min. Then, in order to detect DNA polymorphisms in repetitive motives of glutenin subunits, Denaturing PolyAcrylamide Gel Electrophoresis (DPAGE) was done. After performing PCR operation and denaturing polyacrylamide gel electrophoresis, it was characterized that six ISSR primers used in this research, show good banding polymorphisms in elite mutant lines, standard commercial cultivars and F_2 populations from crosses (Figures 2 and 3).

DISCUSSION AND CONCLUSION

After amino acid sequences alignment of y-type subunits and x-type subunits and based on papers "Shewry and Tatham (1990)(1) and Gianibelli et al. (2001)(2), it was characterized that common repetitive motives of x type HMW glutenin subunits contain hexapeptides "PGQGQQ, GQQPGQ, GQQ(P)GYY, PGQWQQ" and heptapeptides "YPTSP(L,S)Q Q(L), GQGQP(Q)GY, QGQQGQQ" and common repetitive motives of y type HMW glutenin subunits contain hexapeptides "PG(E)QGQQ ،QQP (S,L)GQG, GYYPTS, QQGHYP, GQQS(I)GQ" and heptapeptides "GHYP(L)ASQ and YPTSL(P)QQ" and can be found that hexapeptides "GYYPTS and PGQGQQ" are common. Six polymorphic primers were designed from hexapeptides "PGQGQQ and GQQPGQ" and heptapeptides "QGQQGQQ and YPTSP(L)QQ".

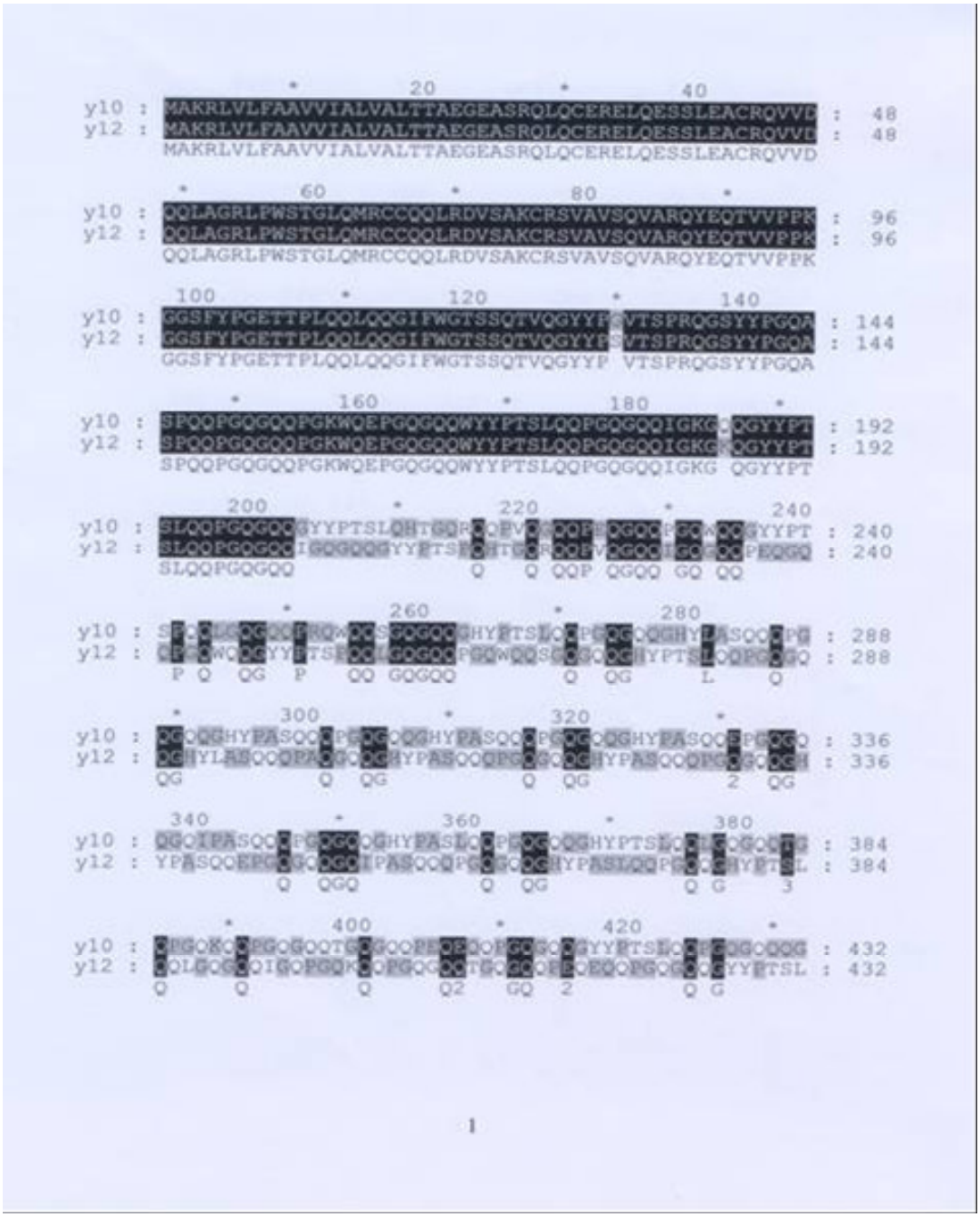

Figure 1. Alignment of amino acid sequences due to y type glutenin subunits in 1D locus.

Table 2. Repetitive motives of six polymorphic primers.

Primer	Repetitive motif
S1	PGQGQQ
S2	PGQGQQ
S3	GQQPGQG
S7	QGQQGQQ
S13	YPTS(L,P)QQ
S14	YPTS(L,P)QQ

Most of deletion or duplication events in glutenin subunits (specially High Molecular Weight) have occurred in glutamine and glycine-rich sequences. Most important insertion and deletion mutations were 185 amino acid sequence insertion of 2.2* subunit and 102 amino acid sequence insertion of x2.2 subunit in position 486 of amino acid sequence and six amino acid sequence deletion "IGQGQQ" in position 203 of y10 subunit. From important point mutations can be pointed to conversion of serine to cysteine in position 118 of x5 subunit and

Table 3. Polymorphic primers information.

Primer	Sequence (5'→3')	Allele
S1	CCA GGA CAA GGG CAA CAA GGR* TAC	Dy10, Dx2, Dx5
S2	CCA GGA CAA GGG CAA CAR CCA GGA	Dx2, Dx5
S3	GGK CAG CAG CCA GGA CAA GGG	Dx2, Dx5
S7	CAA GGG CAA CAA GGK CAG CAG	Dx2, Dx5
S13	TAC CCA ACT TCT CTA CAR CAG	Dy10, Dy12
S14	TAT CCA ACT TCT CCR CAG CAG	Dy10, Dy12, Dx2, Dx5

* In above sequences, R = A,G and K = G,T.

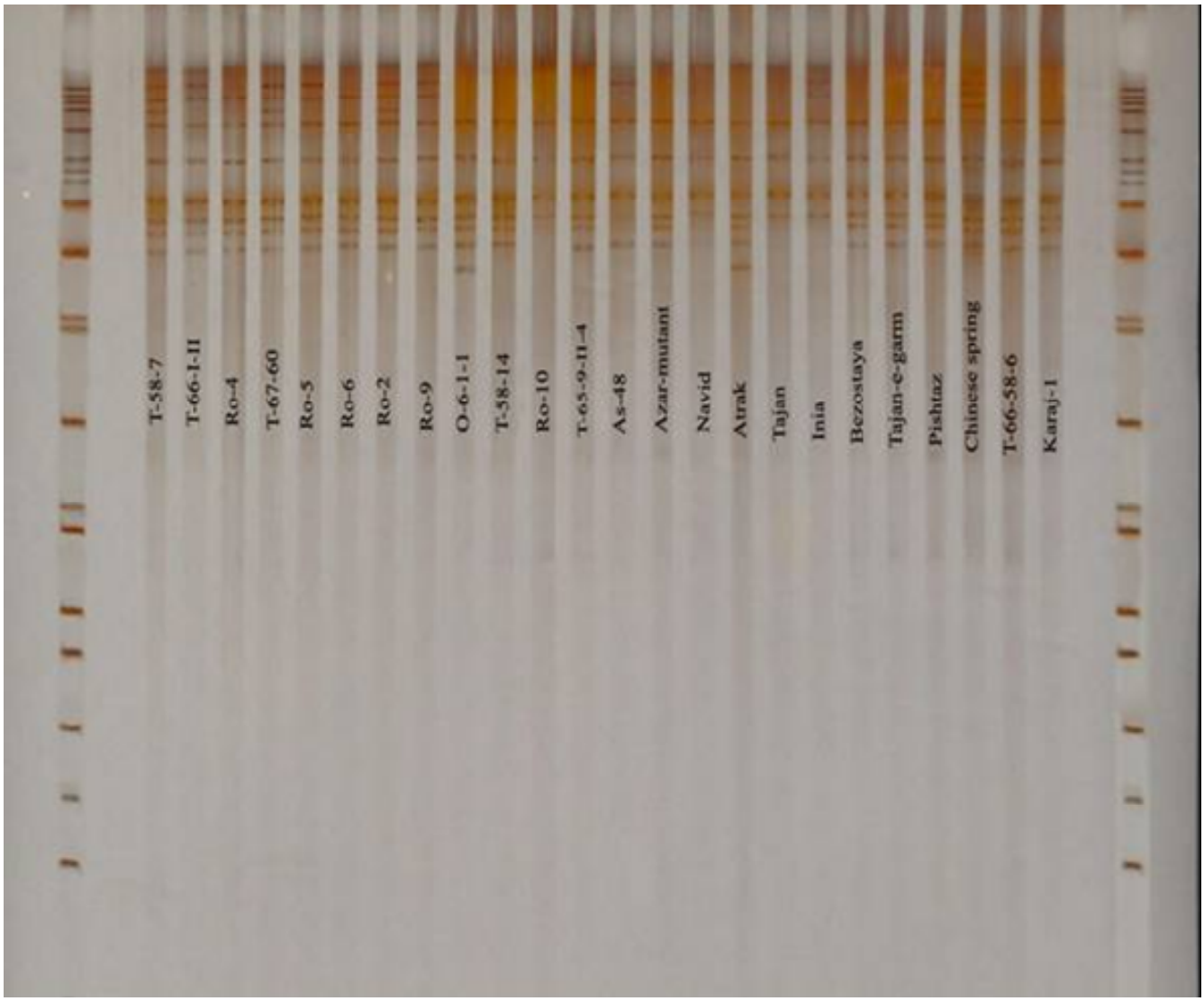

Figure 2. ISSR banding pattern of S1 primer designed from Dy10, Dx2, Dx5 glutenin subunits in some elite mutant lines and standard commercial cultivars.

substitution of glutamine to histidine in position 626 of x5 subunit. In the other hand, most of important point mutations occurred in subunits, were substitution or conversion of amino acids involved in secondary and tertiary structures (α-helix and β-sheet) that have very important role in stability, function and activity of storage proteins.

In view of ISSR banding patterns, it can be found many allelic and banding variations in elite mutant lines and

commercial cultivars and F2 generation. For example, banding patterns of cultivar ‘Atrak’ carrying 5+10 allele and cultivar ‘Chinese spring’ carrying 2+12 allele have several differences. Also, there are several variations between elite mutant lines with different origins. Morever, it was observed between elite mutant lines, commercial cultivars and F2 generations.

By virtue of insertions, deletions and point mutations occurred in genome and after performing physical and chemical expriments on baking quality and PCR test of these mutant lines and commercial cultivars, it was characterized elite mutant lines and commercial cultivars carrying 5+10 allele, have higher and better baking value and also, it can be found cause of superior 5+10 allele to 2+12 allele and other alleles and it's effect level in baking quality of bread wheat. Therefore, in view of good polymorphisms of six designed ISSR primers in elite mutant lines, standard commercial cultivars and F_2 populations from crosses, it was found that these primers are ideal for DNA polymorphisms detection in HMW glutenins subunits of 1D locus.

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