Germplasm enhancement in bread wheat targeting quality characteristics

Hassani ME[1], **Shariflou MR**[2], **Bariana H**[2], **Sharp PJ**[2]

[1]The University of Tehran, Faculty of Agriculture, Department of Horticultural Sciences, Karaj-Iran

[2]The University of Sydney, Plant Breeding Institute Cobbitty, Sydney-Australia

ABSTRACT

The synthetic hexaploid approach was used to introgress a novel high molecular weight glutenin subunit (HMW GS) gene (12.4^t) into bread wheat. Tetraploid wheat *T. turgidum* cv Langdon was crossed with *T. tauschii* accession AUS24092. Immature embryos were rescued and placed onto B5 regeneration media. Hybrid F_1 plants were treated with colchicine. Synthetic hexaploid L/24092 was isolated and used for further crossing and backcrossing programs. Bread wheat cvs Baxter, Sunvale, Sunlin, and Sunbri were crossed with synthetic hexaploid L/24092. Doubled haploid lines were produced using F_1 seeds and phenotyped for the presence of the HMW GS $Dy12.4^t$ protein, using SDS-PAGE of total proteins under reducing conditions. The doubled haploid lines were divided into 17 groups with different combinations of HMW GS, with and without the HMW GS ($Dy12.4^t$) protein for functional studies.

Corresponding author: M. E. Hassani

E.mail: mehassani@ut.ac.ir

Tel/Fax 001198-261-2248721

INTRODUCTION

The progenitors of bread wheat are genetically very broad and dispersed in a wide area of the Middle East, but only a few wild relatives contributed to the bread wheat genome. Studies of *T. tauschii* accessions have shown that the D genome donor of bread wheat contains greater levels of genetic variation than the D genome present in bread wheat in terms of protein genes (Lagudah and Halloran, 1988). Seed storage proteins have the major role in developing flour into cohesive, visco-elastic dough. A major class of storage proteins is polymeric proteins sub-grouped into HMW and LMW glutenin subunits, bound into the polymeric structure of the dough network by a process of covalent bonding (SS) between cysteine residues of polypeptides. The HMW GS are quantitatively minor, play a major role in the functional properties of dough and are the main target for the characterisation and investigation of their properties. HMW GS (x-type and y-type) are encoded by two closely linked genes at the *Glu-1* loci (*Glu-A1, Glu-B1 and Glu-D1)* located on the long arms of homoeologous group 1 chromosomes. Variation in storage proteins especially HMW GS has been extensively studied using SDS-PAGE, with allelic differences found at all three loci (Payne et al. 1984).

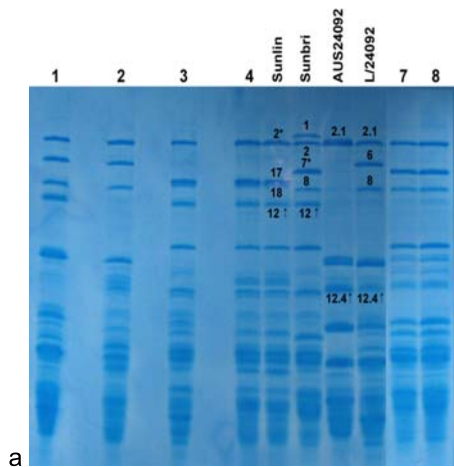
Cultivated bread wheat is known to have restricted variation of desirable genes such as storage protein genes. Wide hybridisation can facilitate the enrichment of the gene pool of bread wheat using synthetic hexaploid approach. This can enrich genetic variability for desirable agronomic characteristics, yield, protein content and resistance to various biotic and abiotic stresses. Wild progenitors of bread wheat, especially those with the D genome can be used as a rich reservoir of valuable genes, especially HMW GS genes. The HMW GS gene (12.4^t) was identified previously in *T. tauschii* accessions AUS24092. The aim of this study was to enhance genetic variability by introducing $Dy12.4^t$ from wild relative D genome to bread wheat, to expand genetic variability in bread wheat using doubled haploid production. This would provide breeding lines, with and without the genes of interest, having the potential to demonstrate the significance of these storage proteins in determining variation in quality characteristics.

MATERIALS AND METHODS

T. turgidum cv Langdon was reciprocally crossed to *T. tauschii* accession AUS24092 to produce the synthetic hexaploid (L/24092), using embryo culture. The derived synthetic hexaploid (L/24092), possessing the HMW GS gene 12.4^t was crossed to Australian cvs Baxter, Sunvale, Sunlin, and Sunbri, resulting F_1 seeds. Wheat x maize system was used to produce doubled haploid lines, using F_1 seeds, according to Laurie and Bennett, 1988). Electrophoresis and total protein analysis was carried out according to Gianibelli et al. (2002).

RESULTS

Synthetic hexaploid L/24092 ($AABBD^tD^t$) was established to introgress the y-type HMW GS gene 12.4^t into Australian bread wheat cultivars. Plant materials (cvs Baxter, Sunvale, Sunlin, Sunbri and synthetic hexaploid L/24092) were grown and crosses were made to establish F_1 seeds for producing doubled haploid lines. A total of 80 DH lines were produced and SDS-PAGE analysis of total proteins of doubled haploid lines revealed 17 combinations of HMW glutenin subunits. The analysis of the DH lines revealed that the lines could be grouped in five sets, allowing the quality evaluation of DH lines for the effects of novel $Dy12.4^t$ in wheat flour (Table 1).



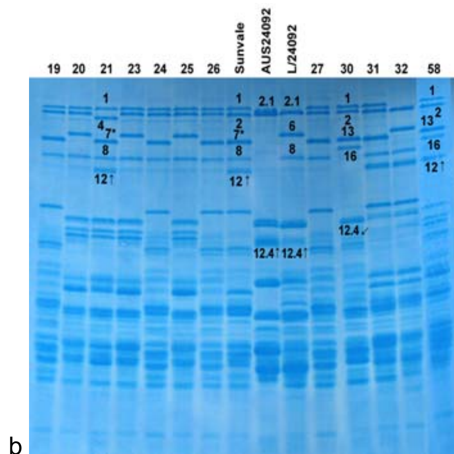


Figure 1 SDS-PAGE analysis of some DH lines and parents in a and b under reducing conditions.

Table 1 Doubled haploid lines derived via wheat x maize system of F_1s resulted from crosses between Australian cvs Baxter, Sunvale, Sunlin, Sunbri, and synthetic hexaploid L/24092. Parents and DH lines were phenotyped using SDS-PAGE analysis of total proteins. H, indicates synthetic hexaploid L/24092; F, indicates Sunlin; D, indicates Sunbri, and C, indicates F_1 (L/24092 x Baxter) top crossed to Sunvale.

Parents		**HMW GS (A)**	**HMW GS (B)**	**HMW GS (D)**
Baxter		1	13+16	2+12
Sunvale		1	7*+8	2+12
Sunlin		2*	17+18	2+12
Sunbri		1	7*+8	2+12
AUS24092		-	-	$2.1^{t}+12.4^{t}$
Langdon		-	6+8	-
L/24092		-	6+8	$2.1^{t}+12.4^{t}$
(L/24092 x Sunlin)	**ID No.**			
HF1	1	-	6+8	2+12
HF1	2	-	6+8	$2.1^{t}+12.4^{t}$
HF1	3	-	17+18	2+12
HF2	4	-	17+18	2+12
Sunbri				
HD1	5	1	7*+8	$2.1^{t}+12.4^{t}$
HD1	6	1	7*+8	$2.1^{t}+12.4^{t}$
HD3	7	-	7*+8	$2.1^{t}+12.4^{t}$
HD3	8	-	7*+8	$2.1^{t}+12.4^{t}$
HD4	9	1	6+8	2+12
HD5	10	-	6+8	2+12
HD5	11	1	6+8	2+12
HD5	12	1	7*+8	$2.1^{t}+12.4^{t}$
HD5	13	-	7*+8	2+12
Sunvale				
HC1	14	1	7*+8	4+12
HC1	15	1	7*+8	$2.1^{t}+12.4$
HC2	16	1	6+8	$2.1^{t}+12.4^{t}$
HC2	17	1	7*+8	$2.1^{t}+12.4^{t}$
HC2	18	-	7*+8	4+12
HC2	19	1	7*+8	$2.1^{t}+12.4^{t}$
HC2	20	1	6+8	$2.1^{t}+12.4^{t}$
HC2	21	1	7*+8	4+12
HC2	22	-	6+8	2+12
HC2	23	1	6+8	$2.1^{t}+12.4^{t}$
HC2	24	1	7*+8	$2.1^{t}+12.4^{t}$
HC2	25	1	6+8	$2.1^{t}+12.4^{t}$
HC2	26	1	7*+8	$2.1^{t}+12.4^{t}$
HC2	27	1	7*+8	$2.1^{t}+12.4^{t}$
HC3	28	1	13+16	$2.1^{t}+12.4^{t}$
HC3	29	1	13+16	$2.1^{t}+12.4^{t}$
HC3	30	1	13+16	$2.1^{t}+12.4^{t}$
HC4	31	1	7*+8	2+12
HC4	32	-	6+8	2+12
HC4	33	-	7*+8	4+12
HC4	34	1	7*+8	2+12
HC4	35	1	7*+8	2+12
HC4	36	-	6+8	4+12
HC4	37	-	6+8	2+12
HC4	38	1	6+8	4+12
HC4	39	1	7*+8	2+12
HC4	40	-	6+8	4+12
HC4	41	-	7*+8	2+12
HC4	42	1	7*+8	4+12
HC4	43	1	6+8	2+12
HC4	44	-	7*+8	4+12
HC4	45	-	7*+8	4+12
HC4	46	-	6+8	4+12
HC4	47	-	6+8	2+12
HC4	48	1	7*+8	4+12
HC4	49	1	6+8	2+12
HC4	50	1	6+8	2+12
HC4	51	-	7*+8	4+12
HC4	52	-	7*+8	2+12
HC4	53	1	7*+8	4+12
HC4	54	-	7*+8	4+12
HC4	55	1	6+8	4+12
HC4	56	-	6+8	2+12
HC4	57	1	6+8	4+12
HC5	58	1	13+16	2+12
HC6	59	1	7*+8	4+12
HC6	60	1	7*+8	4+12
HC6	61	1	7*+8	4+12

HC6	62	1	6+8	4+12
HC6	63	1	7*+8	4+12
HC6	64	1	6+8	4+12
HC6	65	1	7*+8	2+12
HC6	66	1	6+8	2+12
HC6	67	1	7*+8	4+12
HC6	68	1	7*+8	2+12
HC6	69	1	7*+8	2+12
HC6	70	1	7*+8	2+12
HC10	71	1	7*+8	4+12
HC10	72	1	7*+8	4+12
HC10	73	1	6+8	4+12
HC10	74	1	7*+8	4+12
HC10	75	1	7*+8	4+12
HC10	76	1	6+8	2+12
HC10	77	1	7*+8	2+12
HC10	78	-	13+16	4+12
HC10	79	-	13+16	4+12
HC10	80	1	13+16	4+12

Table 2 Groups of DH lines suggested for functional studies.

DH group	No. of Lines	Glu-A1 Subunit	Glu-B1 Subunits	Glu-D1 Subunits
1	7	-	6+8	2+12
	1	-	6+8	2.1^t+12.4^t
2	7	1	6+8	2+12
	4	1	6+8	2.1^t+12.4^t
3	3	-	7*+8	2+12
	2	-	7*+8	2.1^t+12.4^t
4	9	1	7*+8	2+12
	9	1	7*+8	2.1^t+12.4^t
5	1	1	13+16	2+12
	3	1	13+16	2.1^t+12.4^t

DISCUSSION

A synthetic hexaploid approach was used to introgress HMW GS gene (Dy12.4^t) by crossing tetraploid wheat cv Langdon to *T. tauschii* accession AUS24092 followed by embryo rescue and chromosome doubling. The results showed that the objective of genetic transfer is achievable using this approach. However, the rate of embryo germination and response to chromosome doubling is greater when tetraploid wheat was used as female parent.

Homozygous populations can be generated by producing doubled haploid lines using the wheat x maize system (Laurie and Bennett 1986). Homozygous production in one generation not only speeds up the breeding program but also generates lines with genetic variability allowing the analysis for different breeding objectives. Doubled haploid lines produced in this study have different combinations of HMW GS in homoeologous loci. These lines will allow examining the effects of the HMW GS with a small repetitive domain (Dy12.4^t) (Hassani et al. 2005) on functional properties of flour. Doubled haploid lines were genotyped and classified into groups carrying the same HMW GS allow the examination of DH lines for functional studies (Table 2).

SDS-PAGE analysis of DH lines was also identified a new allele located at the *Glu-D1* encoding a HMW GS Dx4. A total of 32 DH lines were identified with different HMW GS combinations possessing 4+12 subunits. Further functional studies can now be carried out comparing HMW GS 2+12 and 4+12. Australian cv Baxter was used in the DH production program possessing HMW GS 1, 13+16, and 2+12. The new allele Dx4 could be from bread wheat cv Baxter which has some biotypes (Bekes, personal communication). Other possibility could be a mutation on Dx2 subunit and a deletion resulted into a new Dx4 subunit. Further characterisation could be carried out to determine the origin of the Dx4 subunit.

REFERENCES

Gianibelli, M.C., Masci, S., Larroque, O.R., Lafiandra, D., and MacRitchie, F. (2002c). Biochemical characterisation of a novel polymeric protein subunit from bread wheat (Triticum aestivum L.). Journal of Cereal Science 35, 265-275.

Hassani, M.E., Gianibelli, M.C., Shariflou, M.R. and Sharp, P.J. (2005). Molecular structure of a novel y-type HMW glutenin subunit gene present in Triticum tauschii. Euphytica 141: 191-198.

Lagudah, E.S., and Halloran, G.M. (1988a). Phylogenetic relationships of Triticum tauschii, the D genome donor to hexaploid wheat 1. Variation in HMW subunits of glutenin and gliadins. Theoretical and Applied Genetics 75, 592-598.

Laurie, D.A., and Bennett, M.D. (1986). Wheat x maize hybridization. Canadian Journal of Genetics and Cytology 28, 313-316.

Laurie and Bennett, 1988. The production of haploid wheat plants from wheat x maize crosses. Theoretical and Applied Genetics. 76:393-397.

Payne, P.I., Holt, L.M., Jackson, E.A., and Law, C.N. (1984). Wheat storage proteins: their genetics and their potential for manipulation by plant breeding. Philosophical Transactions of the Royal Society of Landon. Series B, Biological Sciences 304, 359-371.