DEVELOPMENT AND VALIDATION OF MOLECULAR MARKERS FOR HIGH MOLECULAR WEIGHT GLUTENINS (HMW-GS) IN DURUM WHEAT

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The different types of products derived from wheat require varieties with special characteristics for processing that are mainly based on the properties of dough. Dough rheology is determined by gluten proteins, gliadins and glutenins, whose play a key role in wheat quality. Among the different types of glutenins, low molecular weight glutenins (LMW-GS) are the largest contributor to gluten protein and functional properties in durum wheat by influencing the amount and distribution of gluten polymers. However, the high molecular weight glutenins (HMW-GS) play a no inconsiderable role, accounting up to the 28% of variation in gluten index.

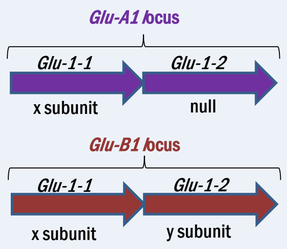
The development of molecular markers from glutenins sequences has had little success due to the high degree of similarity among all genes, the occurrence of many repeated sequences and pseudogenes and the lack of reliable genomic information. However, molecular markers based on competitive allele-specific PCR (KASP) technology have recently started to be successfully applied in wheat and their potential for glutenin genotyping has been assessed in bread wheat.[1]

THE OBJECTIVE OF THIS WORK HAS BEEN THE DEVELOPMENT OF KASP MOLECULAR MARKERS FOR HMW-GS, IN A SET OF DURUM COMMERCIAL VARIETIES AND THEIR VALIDATION IN A COLLECTION OF 200 SPANISH DURUM WHEAT LANDRACES[2] BY COMPARISON WITH SDS- PAGE (SODIUM DODECYL SULPHATE–POLYACRYLAMIDE GEL ELECTROPHORESIS) GRAIN PROFILES.

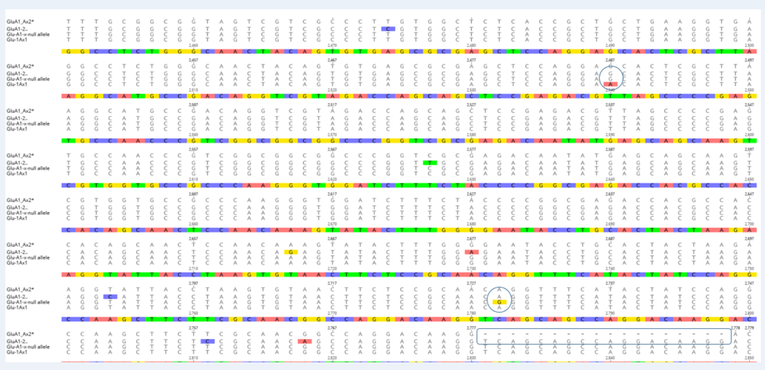
1 GLUTENIN SEQUENCE RETRIVE FROM DATABASES

http://www.ncbi.nlm.nih.gov/genba nk/

	Gene	Accession number	pb	Reference
Glu-A1-x-null	*GluA1-1*	AF145590.1	2537	*De Bustos et al., 2000*
Glu-A1-x-2*	*GluA1-1*	M22208.2	6837	*Anderson, OD y Greene, FC., 1989*
Glu-A1-x-2··	*GluA1-1*	DQ533690.1	4648	*Gobaa et al., 2007*
Glu-A1-x-1	*GluA1-1*	X61009.1	2885	*Halford et al., 1992*
Glu-B1-x-6	*GluB1-1*	KM116481.1	747	*Ravel et al., 2014*
Glu-B1-x-7	*GluB1-1*	DQ537336.1	206063	*Gu et al., 2006*
Glu-B1-x-13	*GluB1-1*	KX454510.1	2391	*n.d.*
Glu-B1-x-14	*GluB1-1*	AY367771.1	4021	*n.d.*
Glu-B1-x-17	*GluB1-1*	AB263219.1	2244	*n.d.*



2 POLYMORPHISM IDENTIFICATION

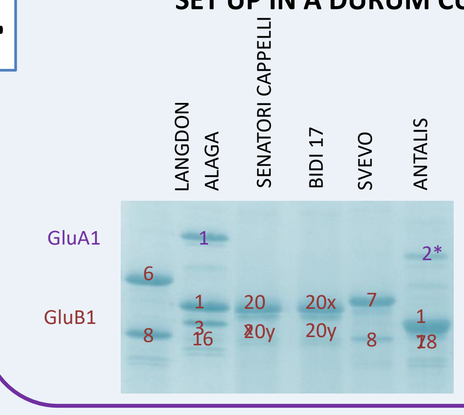


3 KASPR DESIGN

KASP name	HEX	allele	FAM	allele
GluA1x_null	G	null	A	other
GluA1x_1	A	1	G	other
GluA1x_2.1	T	2*	G	2··
GluA1x_2.3	A	2*	G	other
GluB1x_6	T	6	C	other
GluB1x_13	A	13	G	other
GluB1x_7.1	G	other	C	7OE
GluB1x7.2	G	7/17	A	other
GluB1x7_17	C	other	T	7/17
GluB1x14_20	A	14/20	G	other

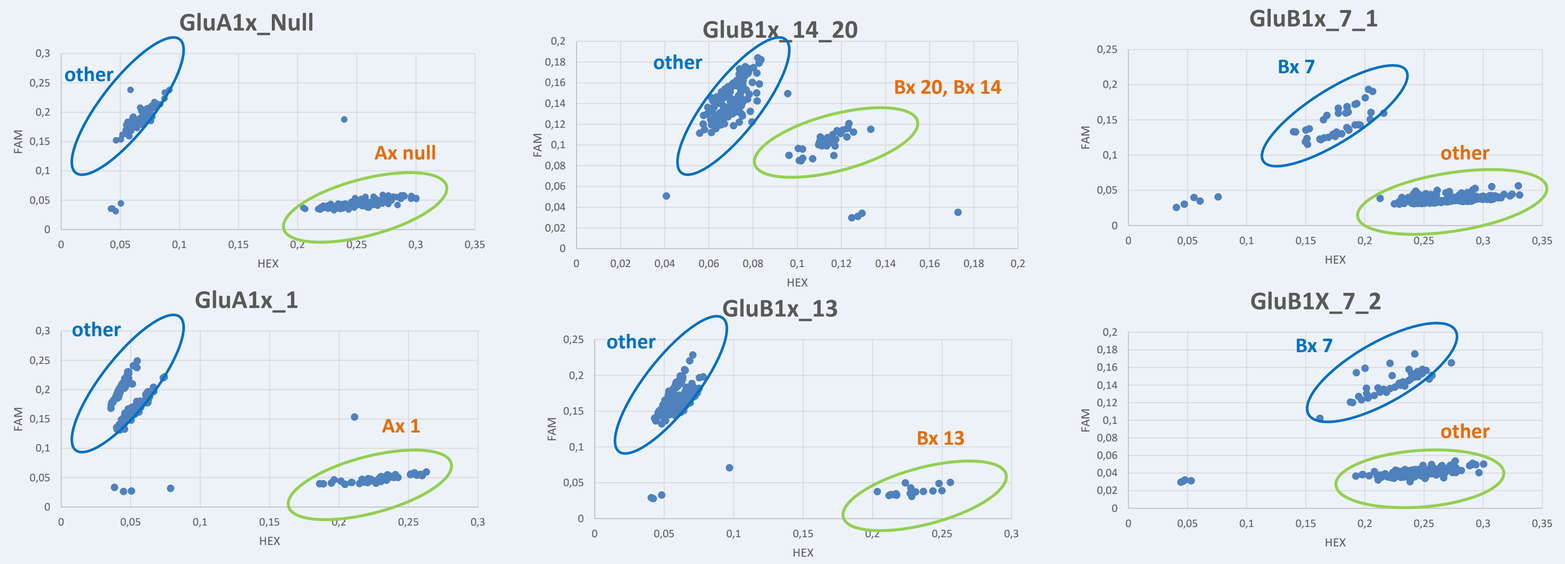
LGC Genomics

4 SET UP IN A DURUM CULTIVARS REFERENCE SET



Roche LC96

5 VALIDATION IN A WIDE DURUM COLLECTION COMPOSED OF 191 LANDRACES AND 23 MODERN VARIETIES



A total of four markers for Glu-A1 alleles and 6 markers for Glu-B1 alleles have been successfully set up

The correspondence with protein-based standard genotyping (SDS-PAGE) was very high, from 97% to 100%.

marker	% accuracy
GluA1x_null	99
GluA1x_1	98
GluA1x_2_1	97
GluB1x_6	100
GluB1x_13	98
GluB1x_7-17	100
GluB1x14_20	97
GluB1x_7_1	98
GluB1x_7_2	98

Glu-A1x	GluA1x_null	GluA1x_1	GluA1x_2.1	GluA1x_2.3
null	G	G	G	G
1	A	A	G	G
2*	A	G	T	A
2··	A	G	G	G

GLU-B1	GluB1x_6	GluB1x_13	GluB1x_7-17	GluB1x14_20	GluB1x_7_1	GluB1x_7_2
Bx6	T	G	C	G	C	A
Bx7	C	G	T	G	G	G
Bx13	C	A	C	G	C	A
Bx14	C	G	C	A	C	A
Bx17	C	G	T	G	G	G
Bx20	C	G	C	A	C	A

THESE ROBUST SET OF MARKERS CONSTITUTE A USEFUL TOOLBOX TO IMPROVE END-USE VALUE AND CAN ALSO ASSIST IN VARIETY IDENTIFICATION AND SELECTION PROCESSES IN DURUM WHEAT BREEDING PROGRAMS.

References

1- Ravel, C., Faye, A., Ben-Sadoun, S., Ranoux, M., Dardevet, M., Dupuits, C., ... & Branlard, G. (2020). SNP markers for early identification of high molecular weight glutenin subunits (HMW-GSs) in bread wheat. *Theoretical and Applied Genetics*, *133*(3), 751-770.

2- Ruiz, M., Giraldo, P., Royo, C., Villegas, D., Aranzana, M. J., & Carrillo, J. M. (2012). Diversity and genetic structure of a collection of Spanish durum wheat landraces. *Crop science*, *52*(5), 2262-2275.

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