Received: 2 September 2018 | Revised: 21 January 2019 | Accepted: 22 January 2019

DOI: 10.1002/cche.10137

RESEARCH ARTICLE

AACCI — Cereals & Grains Association

Effect of *Glu-D1* introgression on dough- and pasta-making quality of durum wheat lines with different glutenin composition and amylose content

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Funding information
NSW Department of Primary Industries; MIUR (Ministry for Education, University and Research)

Abstract

Background and objectives: Durum wheat is mainly used for the production of pasta, but a significant amount is also used for bread and other products worldwide. However, leavened bread made from durum wheat flour or as blends results in bread with lower loaf volume compared to bread made from hexaploid wheat probably due to poor dough strength and/or extensibility. Durum wheat lacks the glutenin subunits important in bread making, in particular the *Glu-D1* subunit 5 + 10. Efforts are being made by researchers to introduce high molecular weight glutenin subunits (HMW-GS), specifically 1, 2*, 17 + 18, 2 + 12, and 5 + 10 and combinations into durum wheat to improve baking quality. Typically, this work has not evaluated pasta-making quality and dual purpose durum wheat is desirable. This work reports the effects of the addition of HMW-GS 2 + 12 and 5 + 10 in various durum wheat backgrounds on dough- and pasta-making quality.

Findings: Durum wheats, Svevo, Svevo partial waxy (null 4A, null 7A, low amylose ~14%), and Lira biotypes 42/45 differing in their LMW-GS as type 1, weak dough, and type 2, strong dough, respectively, were compared with lines having the *Glu-D1* subunit pair 2 + 12 or 5 + 10. For Svevo, the *Glu-B1* 7 + 8 subunits were removed. The absence of 7 + 8 in Svevo reduced the over strong dough strengthening effect, especially from 5 + 10 but also 2 + 12 found previously when 7 + 8 is present. The weak gluten Lira42 genotype benefited from the improved dough strength from 2 + 12/5 + 10, and both Lira biotypes showed much larger effects on dough strength from the *Glu-D1* pairs than with Svevo, a better quality variety. The impacts on pasta were variable depending on the genotype. For Lira42, the presence of 2 + 12 lowered stickiness and cooking loss while stickiness was only reduced in Lira45 with 5 + 10. For Svevo (without 7 + 8), there was little to no impact on pasta quality from the presence of either 2 + 12 or 5 + 10. The very low amylose Svevo (SvLA) pasta quality was improved greatly by 5 + 10 improving firmness and reducing stickiness and cooking loss although still softer than Svevo.

Conclusions: Manipulation of the glutenin composition of durum wheat by introduction of *Glu-D1* subunits 2 + 12 or 5 + 10 in various durum backgrounds had different effects. Generally, dough strength was improved more so in the weaker dough strength genotypes with these subunits and removing *Glu-B1* 7 + 8 from Svevo provided a more balanced dough strength. Pasta firmness in Svevo and Lira with these

subunits was not affected, and there were minimal changes in other pasta properties except an improvement in pasta stickiness in Lira. In the variable amylose Svevo genotypes, the 5 + 10 subunit pair improved pasta firmness of the overly soft low amylose waxy pasta and reduced stickiness.

Significance and novelty: Manipulation of the glutenin subunit composition of durum wheat by introduction of *Glu-D1* subunits affected dough properties, improving dough strength in genotypes with weak gluten. This study found minor impacts on pasta quality allowing the flexibility to develop durum with a better balance of glutenin subunits more suited to bread making without adversely affecting pasta-making quality and acceptability.

KEYWORDS

dough strength, durum wheat, glutenin, pasta quality, waxy

1 | INTRODUCTION

While improvements in bread-making quality of 100% durum flour or a combination of durum and common wheat flour blends is desirable, a dual purpose durum wheat that still possesses good pasta-making quality is preferred. Several studies demonstrated the existence of a quantitative and qualitative linkage between durum wheat quality and low molecular weight glutenin subunits (LMW-GS). This class of seed storage proteins, encoded from genes located on *Glu-3* loci, affects the end-use quality of the durum wheat, especially the group of proteins associated with the locus *Glu-B3* (Payne, Jackson, & Holt, 1984; Pogna, Autran, Mellini, Lafiandra, & Feillet, 1990). Two main allelic variants have been reported, namely LMW-1 and LMW-2. The first is genetically associated with the γ-gliadin 42, whereas the latter with γ-gliadin 45 (Payne et al., 1984). Durum wheat cultivars with the better pasta-making quality possess always the LMW-2 (D'Ovidio & Masci, 2004). It has been suggested that the improved quality attributes to LMW-2 proteins were related to the higher expression and quantity of LMW-GS present in LMW-2 than LMW-1 types (Autran, Laignelet, & Morel, 1987; D'Ovidio, Marchitelli, Ercoli, Cardelli, & Porceddu, 1999). Although Pogna et al. (1990) reported that the positive effects of LMW-2 were additive with the presence of the high molecular weight glutenin subunits (HMW-GS) 7 + 8, a direct involvement of the HMW-GS for pasta quality is not yet clear. In addition, no studies where the *Glu-D1* subunits have been introduced into durum have evaluated pasta technological quality except in our previous work (Sissons, Pleming, Margiotta, D'Egidio, & Lafiandra, 2014). In that study, the 1Dx5+1Dy10 and 1Dx2+1Dy12 were introduced into the Italian durum wheat Svevo, which has the *Glu-B1* 7 + 8 subunit pair present. The addition of 5 + 10 made the dough overstrong and inextensible and the 2 + 12 was not much improved and both failed to improve loaf quality over Svevo. Cooked spaghetti firmness was reduced by the addition of these subunit pairs, and it was suggested that a better balance of high to low molecular weight glutenin subunits is needed to achieve improvements in loaf volume while minimizing impacts on pasta quality (Klindworth et al., 2014).

The objectives of this work were to determine the effect of the addition of HMW-GS 2 + 12 and 5 + 10 from the *Glu-D1* locus of common wheat donor into different durum wheat genotypes with varying HMW-GS, LMW-GS composition and amylose contents on both the dough and pasta technological quality.

2 | MATERIALS AND METHODS

2.1 | Plant material

For details on the plant material used, please refer to Sissons, Pleming, Sestili, and Lafiandra (2019). We have used data from Sissons et al. (2014) in Figures 3 and 6 for Svevo with subunits 7 + 8 and 5 + 10 (Sv7+8, 5 + 10**) and Svevo with 7 + 8 and 2 + 12 (Sv7+8, 2 + 12**) for comparative purposes. Genetically similar sister lines of Sv2+12 (3 and 7) and 5 + 10 (A4 and A6) were evaluated. The SvLA is a full waxy lacking the GBSS proteins encoded at 4A and 7A but the sample was contaminated with normal Svevo seed and this increased the amylose content from ~1–2% to 14.8%.

2.2 | Electrophoretical analyses

Glutenin subunits were extracted and analyzed by SDS-PAGE as described by Gennaro et al. (2012) with modifications. To extract the glutenin subunits, 100 mg of flour was extensively washed with 50% 1-propanol to eliminate the monomeric fraction. The residue was then mixed with 80 mM Tris–HCl pH 8.5, 50% 1-propanol, and 1% DTT (sample/buffer ratio 1:5 w/v) and kept for 30 min at 65°C. After centrifugation (15 min at 10,000 *g*), the supernatant

was recovered and the subunits alkylated with 1.4% (v/v) 4-vinylpyridine. Glutenin subunits were precipitated with 4 volumes of cold acetone and stored overnight at −20°C. The sample was then centrifuged (10,000 *g* for 30 min at 4°C) and rinsed several times with cold acetone. The pellet obtained was resuspended in 40 mM Tris–HCl pH 6.8, 2% SDS, 10% (v/v) glycerol, and 1% DTT for 15 min and separated by one-dimensional gel electrophoresis (SDS-PAGE; $T = 10$, $C = 0.8$). Gels were stained overnight in 12% TCA containing 5% Coomassie-R250 in absolute ethanol (1% v/v) and destained in deionized water.

2.3 | Technological tests—semolina preparation and characterization

Wheat was cleaned, conditioned to a water content of about 16.5%, and left to moisten overnight. Standard milling was performed in a Buhler MLU 202 mill (Buhler, Uzwil, Switzerland) with three breaking and three sizing passages (AACC Method 26-41.01). Semolina protein was determined using in-house calibrations on a NIRSystems model 6,500 spectrophotometer (Foss NIRSystems Inc., Laurel, MD, USA) as a single scan. Semolina moisture was determined by Approved Method 44-15A (AACC, 2010). Semolina color was evaluated by measuring *L** (brightness, 100 = white; 0 = black), *a** (positive value is redness and negative value is greenness), *b** (positive value, yellowness; negative value, blueness), and whiteness index (WI) parameters with a Minolta Chroma meter CR-410 (Biolab Australia, Sydney) taken at three different positions over the sample and the unit provides a mean value which was recorded.

2.4 | Starch analysis of pasta

The pasting properties of uncooked spaghettis were based on a previously published procedure (Aravind, Sissons, Fellows, Blazek, & Gilbert, 2013) measured with a Rapid Visco Analyzer (RVA4, Perten Instruments, Sydney, Australia) interfaced with a computer equipped with Thermocline software. Three replicate runs were used for each sample, and the data are presented as the mean RVA values: peak viscosity (PV), final viscosity (FV), peak time, pasting temperature, trough, setback, and breakdown. Swelling power was measured as described elsewhere (Sharma, Sissons, Rathjen, & Jenner, 2002) in duplicate. Amylose content of pasta was determined by grinding spaghetti strands using a coffee grinder, sieved across a 250μm screen, and assayed using Megazyme kit (amylose/amylopectin; Deltagen Australia, Melbourne, Australia).

2.5 | Dough measurements

The procedures used to assess the semolina dough properties of samples were mixograph, Kieffer rig attachment to the TA.XT2 texture analyzer and Glutopeak, and these have been described previously (Sissons, 2016; Sissons et al., 2014). Data presented and analyzed are the mean of duplicate determinations. The following parameters were measured for mixograph: mixograph peak development time (MPT) and resistance breakdown (RBD), calculated as the change in the value of the curve width at 8 min after peak resistance and expressed as a percentage of the relevant value at the peak resistance. The amount of water used was aimed to attain a peak resistance (height) around the midpoint of the chart, 6.5 ml. For Kieffer rig: peak force (Force), distance and area under the curve (Area) were recorded. A relatively new instrument, GlutoPeak (Brabender, Duisburg, Germany), was used to measure the gluten aggregation properties of the 2014 sample set using the method described previously (Sissons, 2016) which gave an opportunity to compare with the other

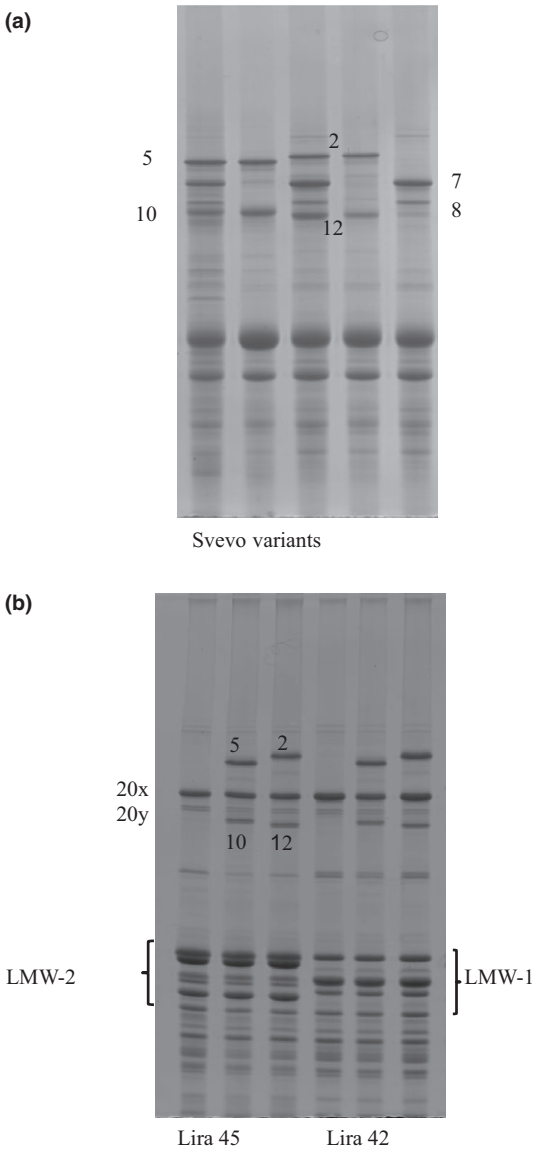

FIGURE 1 SDS-PAGE gels of glutenin subunits extracted from Svevo (a) and Lira (b) genotypes. Subunit pairs are shown

TABLE 1 Glutenin subunit composition at *Glu-1* and *Glu-3* loci of the different durum wheat genotypes

Sample	*Gul-A1*	*Glu-B1*	*Glu-D1*	LMW type	Amylose (%)
Lira42	Null	20		1	27.6
Lira42 2 + 12	Null	20	2 + 12	1	30.2
Lira42 5 + 10	Null	20	5 + 10	1	29.5
Lira45	Null	20		2	31.8
Lira45 2 + 12	Null	20	2 + 12	2	33.6
Lira45 5 + 10	Null	20	5 + 10	2	29.7
Svevo	Null	7 + 8		2	29.3
Sv 2 + 12 (3&7)	Null	null	2 + 12	2	28.4
Sv 5 + 10 (A4&A6)	Null	null	5 + 10	2	31.0
Sv 7 + 8, 2 + 12	Null	7 + 8	2 + 12	2	24.0
Sv 7 + 8, 5 + 10	Null	7 + 8	5 + 10	2	29.2
SvLA	Null	7 + 8		2	14.8
SvLA 5 + 10	Null	7 + 8	5 + 10	2	20.1
SvWx4A	Null	7 + 8		2	27.6
SvWx4A 5 + 10	Null	7 + 8	5 + 10	2	25.3
SvWx7A	Null	7 + 8		2	24.1
SvWx7A 5 + 10	Null	7 + 8	5 + 10	2	26.5

Note. Sv 2 + 12 (3&7) and Sv 5 + 10 (A4&A6) each have two sister lines designated differently.

measures of dough quality. The main indices collected were the peak mixing time (PMT, corresponding to the time to peak torque) and the maximum torque (corresponding to the peak torque occurring as gluten aggregates).

2.6 | Spaghetti making and evaluation

The semolina was processed into dried pasta using a small-scale extruder and high temperature drying cycle, cooked to optimum cooking time (OCT), and assessed for texture (firmness and stickiness), water absorption, and cooking loss as described previously (Sissons et al., 2014). The mean of three measurements is reported for firmness peak height (Firm-PH, the maximum force achieved during the compression), stickiness peak height (S-PH), and area under the curve (S-Area). For water absorption (WABS, calculated as the change in weight after cooking expressed as a percentage of the uncooked weight) and cooking loss (CL), measurements were performed in duplicate. Uncooked pasta brightness (DPL*), redness-greenness (DPa*), yellowness (DPb*), and whiteness index (DPWI) were measured with a Minolta Chroma meter CR-410 (Biolab Australia, Sydney) using 7-cm-length pieces of spaghetti strands aligned to minimize air spaces.

2.7 | Determination of glutenin molecular weight distribution

Size exclusion high-performance liquid chromatography (SE-HPLC) was performed to assess the ratio of polymeric-to-monomeric protein (P/M), glutenin-to-gliadin (Glu/Gli), and the percentage of unextractable polymeric protein (UPP%) but only in the 2014 set where a composite across field replicates was analyzed. The procedure of Sissons, Egan, and Gianibelli (2005) was applied, except the HPLC equipment used was a Waters 1525 Binary HPLC Pump with Waters 2,996 Photodiode Array Detector running Empower 2 software. Two extractions per sample were prepared, and for each extraction, duplicate injections were made. Data presented were the mean of the four measurements per sample.

2.8 | Statistical methods

Only a field composite sample for each genotype was obtained from the 2013 season, and the data could not be statistically analyzed. For the 2014 season, there were three field replicate samples and the data for semolina/dough properties (protein, moisture, color, swelling power, mixograph, Kieffer rig, and GlutoPeak) were analyzed using field replicate as block in the balanced one-way analysis of variance (ANOVA). For spaghetti making, it was necessary to prepare composite samples from the field replicates in 2014 to ensure sufficient pasta for further testing and reduce the number of analyses to be manageable. The composite samples from 2013 were used for pasta making. Two pasta batches were prepared per genotype and pasta analyses were

TABLE 2 Dough characteristics of the different durum wheat genotypes, 2014 season

	Mixograph		Kieffer Rig			Glutopeak	
Genotype	MPT (min)	RBD	Force (g)	Distance (mm)	Area (g/s)	PMT (s)	Torque (AU)
Lira42	1.7^{a}	88^{a}	16.9^{a}	43.7	63.8^{a}	91^{ah}	7.7
Lira42 2 + 12	4.1^{abd}	68^{ad}	35.0^{bc}	45.4	166.3^{b}	118^{ac}	41.7
Lira42 5 + 10	7.4^{c}	12^{b}	35.8^{bc}	39.1	153.9^{be}	210^{b}	32.6
Lira45	2.6^{ab}	73^{ad}	22.7^{adfg}	43.4	94.6^{c}	132^{cf}	24.8
Lira45 2 + 12	6.6^{c}	27^{b}	31.2^{bef}	40.5	136.6^{bd}	182^{d}	34.6
Lira45 5 + 10	7.6^{c}	25^{b}	39.6^{c}	34.1	158.5^{b}	281^{e}	24.7
Svevo	4.1^{bd}	66^{a}	25.8^{defgh}	42.9	109.8^{cd}	114^{ac}	32.0
Sv 2 + 12(3)	4.2^{bd}	46^{dc}	26.8^{defgh}	37.8	107.6^{cd}	171^{df}	27.6
Sv 2 + 12(7)	4.1^{bd}	46^{dc}	29.2^{f}	46.0	133.1^{bd}	147^{f}	31.1
Sv 5 + 10(A4)	3.8^{ad}	46^{dc}	34.5^{bc}	35.1	120.4^{de}	300^{e}	17.9
Sv 5 + 10(A6)	5.3^{bdc}	59^{adc}	28.9^{fh}	41.0	113.4^{cd}	296^{e}	16.9
SvLA	4.1^{bd}	67^{ac}	18.4^{a}	49.7	88.9^{ac}	62^{gi}	53.9
SvLA 5 + 10	5.6^{dc}	46^{cd}	27.9^{fh}	40.4	122.4^{de}	111^{ac}	38.6
SvWx4A	3.6^{ad}	66^{ac}	20.4^{ag}	42.6	84.6^{ac}	113^{ac}	29.8
SvWx4A 5 + 10	6.2^{cd}	22^{bd}	33.6^{bc}	46.1	141.5$_{bd}$	157^{f}	40.6
SvWx7A	2.1^{a}	82^{a}	15.4^{a}	39.7	50.7^{a}	80^{hi}	28.9
SvWx7A 5 + 10	6.2^{cd}	40^{cd}	30.9^{bh}	41.5	139.4^{bd}	230^{b}	43.3
Significance	<0.001	<0.001	<0.001	ns	<0.001	<0.001	<0.001
LSD	2.6	32	7.9	9.7	34.8	26	5.0

Note. MTP: mixograph peak time; RBD: mixograph resistance breakdown; PMT: Glutopeak peak mixing time; numbers with alike letters in the same column are not statistically different, $p < 0.05$.

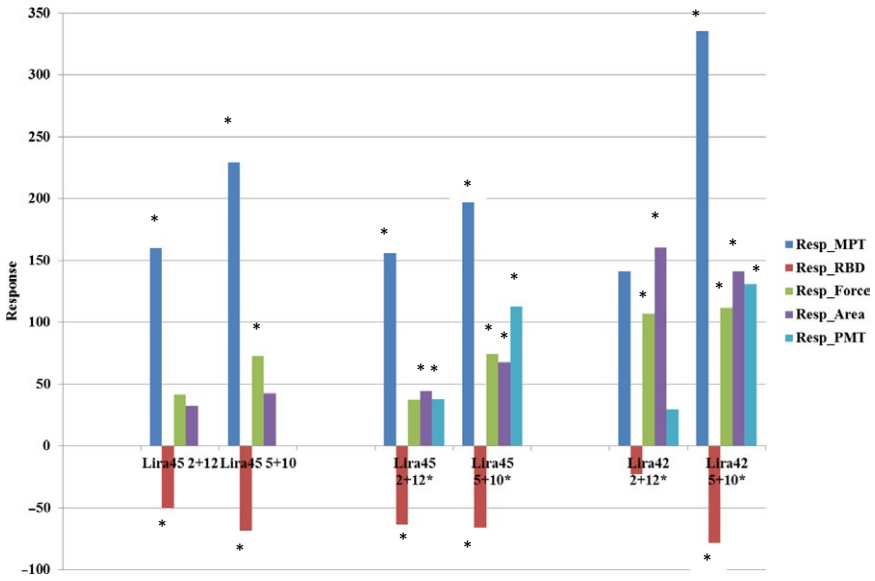

FIGURE 2 Dough property responses for Lira genotypes, combined 2013/2014 and 2014 (*) seasons. Response defined as % change relative to control. The * over bars shows where the means are significantly different to respective control genotypes for that set [Color figure can be viewed at wileyonlinelibrary.com]

performed on these duplicate samples (except color) and analyzed by ANOVA using pasta batch as a block term in ANOVA. Analysis was performed using Statistical Analysis System (GenStat 11.1, VSN International Ltd.) software. Means are compared to test for significant differences ($p < 0.05$) using the least significant difference statistic (LSD).

3 | RESULTS AND DISCUSSION

3.1 | One-dimensional electrophoretic separation (SDS-PAGE) of glutenin subunits and the glutenin molecular weight distribution

The SDS-PAGE patterns of glutenins for the genotypes are shown in Figure 1, and the subunit composition is summarized

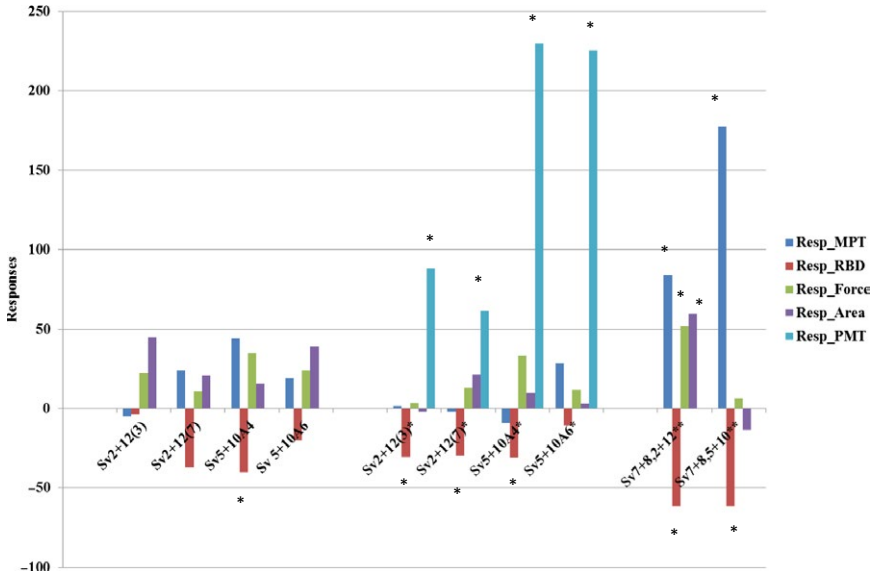

FIGURE 3 Dough property responses for Svevo genotypes combined 2013/2014, 2014 (*), and 2010 (**) seasons. Response defined as % change relative to control genotype. The * over bars shows where the means are significantly different to respective control genotypes for that set [Color figure can be viewed at wileyonlinelibrary.com]

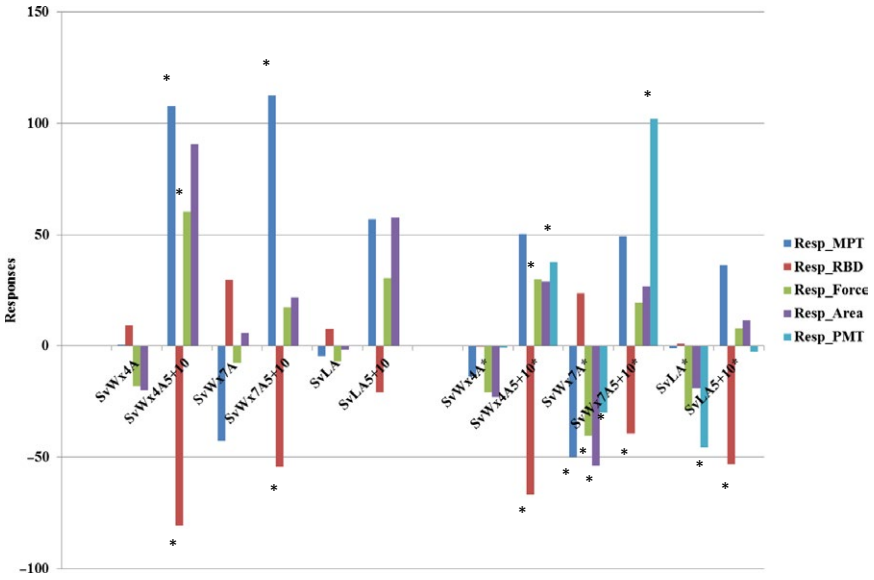

FIGURE 4 Dough property responses for Svevo waxy genotypes, combined 2013/2014 and 2014 (*) seasons. Response defined as % change relative to control genotype. The * over bars shows where the means are significantly different to respective control genotypes for that set [Color figure can be viewed at wileyonlinelibrary.com]

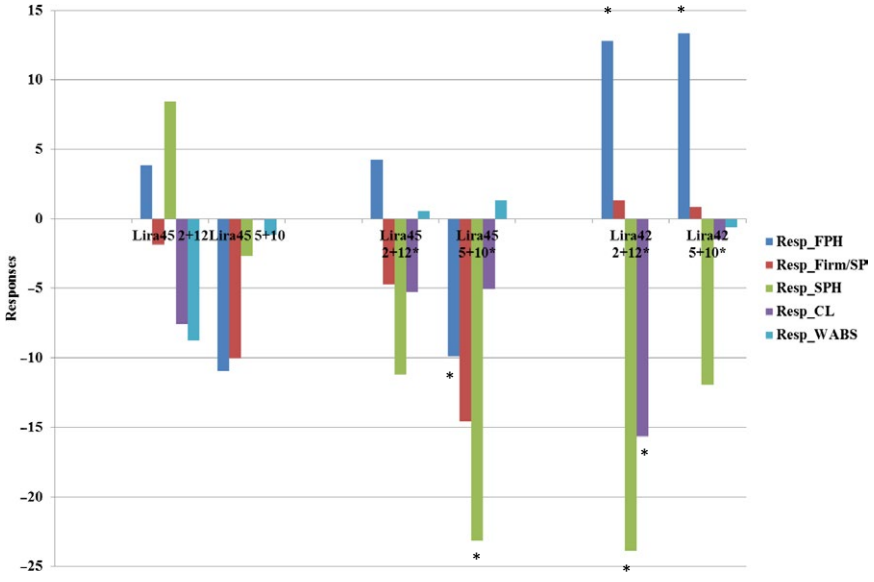

FIGURE 5 Pasta quality responses for Lira genotypes, combined 2013/2014 and 2014 (*) seasons. Response defined as % change relative to control genotype. The * over bars shows where the means are significantly different to respective control genotypes for that set [Color figure can be viewed at wileyonlinelibrary.com]

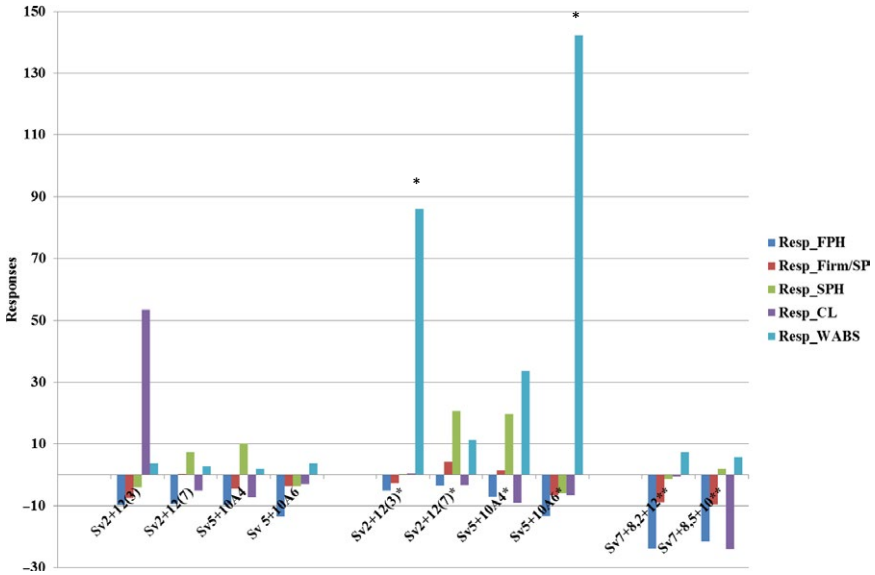

FIGURE 6 Pasta quality responses for Svevo genotypes combined 2013/2014, 2014 (*), and 2010 (**) seasons. Response defined as % change relative to control genotype. Note details of 2010 samples are reported in Sissons et al. (2014). The * over bars shows where the means are significantly different to respective control genotypes for that set [Color figure can be viewed at wileyonlinelibrary.com]

in Table 1. These clearly show the presence of the 2 + 12 and 5 + 10 in Svevo with and without 7 + 8 (Figure 1a) and in the Lira biotypes that differ in their LMW-GS pattern as either LMW-1 or LMW-2 (Figure 1b). The presence of the subunits 5 + 10 or 2 + 12 in Svevo or Lira background permitted to investigate the influence of these subunits on technological properties compared to their respective controls: Lira42, Lira45, Svevo, SvLA, SvWx4A, and SvWx7A. The two biotypes of Lira with the same HMW-GS permitted to evaluate the effects of their different LMW-GS and the interaction with the new HMW-GS allowing comparison of the influence of LMW-GS type with and without *Glu-D1* subunits. For the lower amylose genotypes (Table 1), the only effect was the presence or absence of the *Glu-D1* subunit 5 + 10 but also with the effect of varying amylose content (14.8%–27.6%).

The impacts of the *Glu-D1* subunits on the glutenin polymer size distribution were measured using SE-HPLC, and key parameters are presented in Table S1 for 2014 season samples only. Weak dough is associated with high levels of extractable proteins (Gupta, Khan, & MacRitchie, 1993; Ohm, Hareland, Simsek, & Seabourn, 2009), whereas strong dough is associated with more unextractable polymeric proteins (UPP%). The presence of the 2 + 12 or 5 + 10 subunit pairs in the gluten composition was apparent in the changes in the P/M, Glu/Gli, and UPP% relative to control genotypes. These subunit pairs increased the P/M, Glu/Gli, and UPP% values except for SvWx7A 5 + 10 having the same P/M and Glu/Gli ratios as SvWx7A but a higher UPP%. The impact of 5 + 10 on UPP% was greater than 2 + 12 across all the genotypes. These data show that the inclusion of these subunits led to a greater amount of the larger polymeric glutenin (higher UPP%) probably due to additional disulfide bond formation, leading to dough that shows more resistance to mixing with higher dough strength as described below in Section 3.2. Increased glutenin-to-gliadin ratio has been reported in lines having a 5 + 10 inclusion (Ammar, Lukaszewsky, & Banowetz, 1997; Klindworth et al., 2014).

3.2 | Comparison of the effect of *GluD-1* subunit addition on dough and pasta properties across seasons and genotype backgrounds

3.2.1 | 2014 Season

Semolina protein content varied from 9.7% to 13.6% averaging 10.7% which is considered low for commercial purposes. For comparing data affected by protein content, it is important there is not too much variation between samples as this can impact on measurements like pasta firmness and torque but for the Lira and Svevo sets, the genotypes have similar protein, varying only 1.3% and 1%, respectively, while the waxy set was more variable, up to 2.4% (Table S1). Higher swelling power was found in SvLA and slightly elevated in the partial waxy types (SvWx4A, SvWx7A) relative to Svevo. With increased amylopectin starch gels swell more (Sharma et al., 2002; Tester & Morrison, 1990). The semolina from the Lira variants had slightly inferior yellowness compared to Svevo and the low amylose genotypes (except SvWx7A), while the waxy variants were less bright. There are no significant differences in whiteness between the samples (Table S1).

The dough properties of the samples were assessed by three methods as there is no single method that can best predict end-use quality (AbuHammad, Elias, Manthey, Alamri, & Mergoum, 2012). A comparison of selected mixograms showing clear visual differences and effects of the 2 + 12 and 5 + 10 inclusions in the different genetic backgrounds is shown in Figures S1, S2. When 2 + 12 or 5 + 10 was added

TABLE 3 Pasta cooking properties of the different durum wheat genotypes, 2014 season

Genotype	OCT (min:sec)	Firm-PH (g)	Firm-PH/protein	S-PH (g)	S-Area (g/sec)	CL (%)	WABS	DPL*	DPa*	DPb*	DPWI
Lira42	8.15	383^{a}	39.7^{ad}	22.6^{a}	13.4^{ag}	5.0^{a}	161^{a}	67.7	2.0	22.9	−23.1
Lira42 2 + 12	8.45	432^{be}	40.5^{acd}	17.2^{bc}	11.3^{bde}	4.4^{b}	161^{a}	70.6	1.3	25.0	−28.6
Lira42 5 + 10	8.15	434^{b}	41.3^{ace}	19.9^{abc}	13.2^{ag}	4.6^{ab}	160^{ac}	71.7	1.6	23.8	−25.9
Lira45	8.15	453^{ef}	46.1^{bc}	21.6^{ab}	12.9^{abg}	4.7^{abc}	159^{ab}	66.4	1.9	21.8	−20.4
Lira45 2 + 12	8.15	472^{f}	44.0^{c}	19.2^{abc}	13.1^{ag}	4.5^{b}	160^{ac}	71.3	1.9	25.6	−30.2
Lira45 5 + 10	8.30	408^{a}	38.5^{adf}	16.6^{c}	8.4^{c}	4.5^{b}	161^{a}	71.1	1.9	22.0	−21.7
Svevo	8.30	442^{be}	38.3^{adf}	19.2^{abc}	9.9^{cd}	5.3^{a}	157^{b}	67.6	2.2	25.9	−29.5
Sv 2 + 12(3)	8.45	420^{bc}	37.3^{df}	19.3^{abc}	11.5^{bd}	5.4^{a}	161^{a}	68.5	2.3	25.5	−29.0
Sv 2 + 12(7)	8.00	426^{bc}	41.5^{ace}	23.2^{a}	12.8^{abg}	5.2^{ac}	157^{b}	67.2	2.1	26.0	−29.5
Sv 5 + 10(A4)	7.45	410^{c}	39.1^{def}	23.0^{a}	12.0^{ae}	4.9^{a}	158^{bc}	69.5	2.0	22.8	−23.3
Sv 5 + 10(A6)	8.15	383^{a}	36.0^{f}	18.1^{bc}	11.4^{bde}	5.0^{a}	164^{d}	68.8	2.2	23.1	−23.8
SvLA	8.15	225^{h}	16.1^{g}	21.3^{ab}	17.6^{f}	5.9^{d}	166^{d}	62.0	4.4	26.0	−27.3
SvLA 5 + 10	8.15	341^{g}	30.1^{h}	21.3^{ab}	14.1^{g}	4.8^{abc}	164^{d}	62.4	4.5	24.2	−24.1
SvWx4A	8.15	400^{ac}	35.4^{f}	21.3^{ab}	12.3^{ab}	4.8^{abc}	164^{d}	64.4	3.6	25.2	−26.9
SvWx4A 5 + 10	8.15	341^{g}	28.0^{h}	19.2^{abc}	12.9^{ab}	ns	ns	61.8	4.5	24.2	24.0
SvWx7A	8.45	453^{e}	41.4^{ace}	18.9^{c}	13.0^{a}	4.9^{a}	158^{bc}	66.8	3.6	23.2	−23.5
SvWx7A 5 + 10	8.15	511^{i}	39.2^{def}	18.8^{c}	12.7^{abg}	4.3^{b}	151^{e}	62.2	4.9	23.2	−22.3
Significance		<0.001	<0.001	ns	<0.001	<0.001	<0.001				
LSD		26	3.6	4.5	1.7	0.4	3				

Note. ns: no sample for testing; Firm-PH: cooked firmness peak height; S-PH: stickiness peak height; S-Area: stickiness area; CL: cooking loss; WABS: water absorption; DPL*: dry pasta lightness; DPa*: dry pasta redness; DPb*: dry pasta yellowness; DPWI: dry pasta whiteness index. Numbers with alike letters in the same column are not statistically different, $p < 0.05$.

to Svevo with 7 + 8 present, very strong mixograms were obtained (long mixing time, wide swings of the pin mixer reflected in the uneven mixogram, reported in Sissons et al., 2014) compared to Svevo 2 + 12/5 + 10 without the 7 + 8 present whose profiles were more like Svevo. For Glutopeak, larger values of peak mixing time indicates higher dough strength while torque is not correlated and affected by protein content (Sissons, 2016). Significant differences were found between the samples for all dough measures except Distance (Table 2). For the Lira variants, Lira42 has weaker dough than Lira45 having shorter mixograph peak time (MPT), lower Force, Area, and Glutopeak peak mixing time. Adding 2 + 12 to Lira42 significantly increased dough strength indicators like Force, Area, and Glutopeak peak mixing time but not MPT and resistance breakdown. However, in Lira45 the addition of 2 + 12 significantly increased MPT, Area, and Glutopeak peak mixing time and decreased resistance breakdown. These impacts can be captured as response (% change, Figure 2) showing a greater effect of 2 + 12 in Lira42 than in Lira45. The presence of 5 + 10 increased dough strength even further than 2 + 12 in both biotypes compared to its parent, with differences noted in the various dough measures depending on genotypes (Table 2, Figure 2).

Inclusion of the 2 + 12 into Svevo in the two sister lines did not show significant differences except for resistance breakdown and Glutopeak mixing time that were, respectively, reduced and increased relative to Svevo. When 5 + 10 was added, a similar impact was seen with significantly longer Glutopeak peak mixing times than in Sv 2 + 12 but other dough measures were similar. The only inconsistency was in Sv 5 + 10 (A6) for resistance breakdown. These data show that the different methods to assess gluten strength do not always discriminate the material equally. Possibly, the Glutopeak method might be a more suitable indicator of dough strength as this provided a clearer separation of the effects of the 2 + 12/5 + 10 inclusion.

Comparing Svevo with the SvLA and two partial waxy, SvWx7A was weakest with the lowest values for MPT (and a profile with narrower width, Figure S2), Force, and Glutopeak peak mixing time although SvLA also had lower Glutopeak peak mixing time than Svevo (Table 2). This shows that Svevo waxy types despite having identical glutenin composition and similar protein contents have quite different dough rheological properties. This effect has been reported previously (Gianibelli, Sissons, & Batey, 2005; Jonnala, MacRitchie, Smail, & Seabourn, 2010). The impact of the 5 + 10 on each waxy type generally strengthened the dough with significant changes in MPT (SvWx7A 5 + 10 only), resistance breakdown (but not for SvLA 5 + 10), a mixogram more indicative of stronger dough (Figure S2), Force, and Glutopeak peak mixing time with specific parameters changing depending on the waxy type.

3.2.2 | Pasta technological properties of genotypes 2014 season

Pasta cooking time was a narrow range from 7 min 45 s to 8 min 45 s. There were significant differences in firmness (peak height) in the samples varying from 225 to 511 g; however, as protein content also influences firmness, this is expressed to take this into account as Firm-PH/protein (Table 3). For Lira42, the addition of 2 + 12 or 5 + 10 significantly

TABLE 4 Mean values for selected dough and pasta characteristics of genotypes in common across both seasons

Genotype	Protein (14%mb)	MPT (min)	RBD	Force (g)	Firm-PH (g)	Firm-PH/Protein (g/s)
Lira 45	9.6^{a}	2.3^{a}	75^{af}	22.6^{acd}	456^{ab}	47.3^{a}
Lira 45 2 + 12	10.2^{ab}	6.0^{bcd}	37^{bc}	32.0^{ab}	474^{a}	46.3^{ab}
Lira 45 5 + 10	9.6^{a}	7.6^{c}	24^{c}	39.1^{b}	406^{be}	42.2^{ab}
Svevo	11.0^{b}	3.5^{ae}	68^{adf}	21.4^{cd}	479^{a}	43.7^{ab}
Sv 2 + 12(3)	10.7^{abc}	3.3^{ae}	66^{adef}	26.1^{acd}	432^{abcf}	40.5^{b}
Sv 2 + 12(7)	9.9^{a}	4.4^{abe}	43^{bd}	23.7^{ac}	434^{abcf}	43.7^{ab}
Sv 5 + 10(A4)	10.3^{ab}	5.1^{def}	41^{be}	28.8^{ac}	432^{abcf}	42.0^{ab}
Sv 5 + 10(A6)	9.9^{ab}	4.2^{abe}	54^{ab}	26.4^{acd}	414^{abcf}	41.9^{ab}
SvLA	11.9^{c}	3.4^{ae}	73^{af}	19.9^{cd}	273^{d}	23.0^{c}
SvLA 5 + 10	11.4^{abc}	5.5^{cef}	54^{ab}	27.9^{ac}	370^{ef}	32.6^{d}
SvWx4A	10.5^{ab}	3.5^{ae}	74^{af}	17.5^{de}	454^{ab}	43.1^{ab}
SvWx4A 5 + 10	11.3^{abc}	7.3^{cf}	13^{c}	34.2^{b}	381^{f}	33.6^{d}
SvWx7A	10.6^{ab}	2.0^{a}	88^{f}	19.7^{cd}	472^{a}	44.7^{ab}
SvWx7A 5 + 10	12.8^{d}	7.5^{cf}	31^{bc}	25.1^{ace}	556^{g}	43.5^{ab}
LSD	1.3	2.4	25.2	9.4	56.2	6.1
p value	0.004	0.002	<0.001	0.010	<0.001	<0.001

increased F-PH with no difference between these two, but not when expressed per unit protein basis, whereas for Lira45, 2 + 12 firmness peak height appears to be higher than Lira45 but is lower after correcting for protein. The 5 + 10 in Lira45 decreased firmness significantly. Pasta stickiness was reduced significantly in Lira42 2 + 12 compared to Lira42 but this was not significant in Lira45 2 + 12 compared to its control. While 5 + 10 tended to lower stickiness, it was only significant in Lira45 5 + 10 reducing stickiness much more. A reduction in stickiness is a desirable consumer trait. Comparing Lira42 or Lira45 with the 2 + 12/5 + 10 presence, there were no differences in cooking loss except for Lira42 2 + 12 being lower and no differences in water absorption in the Lira genotypes. There were subtle differences in the color space between the Lira biotypes with Lira45 being duller and less yellow than all other Lira genotypes.

For the Svevo set, neither 2 + 12 nor 5 + 10 affected pasta firmness peak height after protein correction (Table 3). This is in contrast to reductions in firmness reported previously (Sissons et al., 2014) in Sv7+8 2 + 12/5 + 10 genotypes (Figure 6). This suggests that removing the 7 + 8 in Svevo helped to reduce the negative impact of the 2 + 12/5 + 10 on pasta firmness. Inclusion of either 2 + 12 or 5 + 10 in Svevo (lacking 7 + 8) had no clear effect on pasta stickiness, cooking loss, and water absorption. There were only small differences in color of the dry pasta between the Svevo genotypes.

For the lower amylose genotypes compared to Svevo, SvLA had significantly lower firmness, while SvWx7A and SvWx4A had equivalent firmness to Svevo when corrected for protein (Table 3). It is known that pasta made from waxy durum wheat (similar to our SvLA sample) is inferior and softer (Grant, Doehlert, McMullen, & Vignaux, 2004), and our results are consistent. Pasta firmness benefited from having the 5 + 10 in SvLA but not in SvWx7A, while the opposite occurred for SvWx4A which is difficult to explain. Data on the stickiness peak height showed no differences in the genotypes; however, the work of adhesion (S-Area) showed that most of the waxy types had higher values than Svevo indicating stickier pasta. Lowering the amylose content of durum wheat leads to increased pasta stickiness (Gianibelli et al., 2005; Grant et al., 2004). The 5 + 10 inclusion had no impact on pasta stickiness in the partial waxy but significantly lowered it in SvLA (S-Area only). Also, cooking loss was reduced significantly in SvLA 5 + 10 compared to SvLA (which was higher than Svevo) and in SvWx7A 5 + 10 compared to SvWx7A. Comparing water absorption of the waxy samples with Svevo, SvWx7A was equivalent and SvWx7A 5 + 10 was significantly lower, while SvWx4A, SvLA, and their 5 + 10 were all higher in WABS. In practice, these differences are not large and this range is typically found in durum pasta. Dry pasta colors show differences between samples in terms of all three parameters with the lower amylose genotypes being more dull (lower L^*), green (increased positive a^*), and having similar yellowness to Svevo types but more yellow than the Lira biotypes. It is possible that the inclusion of 5 + 10 allows a stronger gluten matrix to form during dough development (Table 2), and this helps to improve firmness and lowers cooking loss and amylose leaching leading to a lower of stickiness compared to SvLA (Table 3). In the SvWx7A 5 + 10, while the firmness was not different to SvWx7A, its cooking loss and water absorption were reduced supporting this possibility. This was not the case in SvWx4A 5 + 10 where firmness actually decreased. This cannot be easily explained.

3.2.3 | Combining both season's data

To combine the 2013 and 2014 data, the mean of the field replicate values from the 2014 season was calculated, and for genotypes and measurements in common across the two seasons, an ANOVA was performed using year as a block term. The results are presented in Table 4 showing only significant genotype effects. For protein, within each of the three sets there is minimal variation in the Lira and Svevo sets but more variability in the waxy/partial waxy set mostly due to the much higher semolina protein in SvWx7A 5 + 10. Clearly, the presence of the extra subunit pairs had no effect on protein content. In Lira45, both 2 + 12 and 5 + 10 increased MPT and reduced RBD making the dough stronger as noted in 2014 but not in the Svevo background except for Sv5+10(A4) with lower RBD. More differences were noted in MPT and RBD in the 2014 data. In the waxy set, 5 + 10 increased MPT and reduced RBD only in the two partial waxy. The Kiefer rig Force values did not increase significantly in Lira45 2 + 12 compared to Lira45 but the 5 + 10 did, consistent with the mixograph data and the 2014 data. Again, Force in the Svevo set showed no differences consistent with the mixograph data. Only 5 + 10 in SvWx4A increased Force as in 2014 data. This suggests the mixograph is more sensitive to changes in dough properties than the Kieffer rig. For the pasta firmness, as this is affected by protein content ($r = 0.6$–0.9), an adjustment is made. While 2 + 12 in Lira45 made firmer pasta than Lira45 5 + 10, this affect was not significant when protein adjustment was made (Table 4). In the Svevo set, there were no differences in pasta firmness with the subunit additions as noted largely in the 2014 data. The SvLA had the lowest firmness of all the samples as noted elsewhere, while the two partial waxy had similar firmness to Svevo. Interestingly, the 5 + 10 in SvWx4A reduced firmness significantly but greatly increased firmness in SvLA.

To compare the effects on dough properties across seasons and genotypes, data were expressed as a response defined as percentage change in absolute value relative to each respective control genotype. This allows comparison

of the impacts across the different genotypes and these are summarized in Figures 2–4 where the first set of bars is the combined 2013 + 2014 mean values and the others are 2014 separate year effects (mean 3 field replicates) and 2010 data, where appropriately taken from Sissons et al. (2014). For Lira set, the largest responses in dough properties were mainly in Lira42 possibly because this is the weaker of the biotypes due to presence of the LMW-1 and so benefits more from the *Glu-D1* presence than in Lira45. Adding the 2 + 12 led to significantly increased dough strength in terms of increases in mixograph peak time and decreases in resistance breakdown in Lira45 2 + 12 but not in Lira42 2 + 12. However, Force showed less sensitivity with no increase in Lira45 2 + 12 in both seasons and 2014 alone but an increase in Lira42 2 + 12. Adding the 5 + 10 led to even greater responses than 2 + 12 produced across all dough measures, and this was reflected in an increase in MPT, Force, Area, PMT, and decreased RBD. This shows the benefits of adding these *Glu-D1* subunits to relatively weak genotypes having HMW-GS 20 and/or LMW-1 thought to be associated with weaker dough (Sissons, 2008). The greater response to these subunit additions in Lira42 could likely be due to having the LMW-1 compared to the LMW-2 type in Lira45.

Previous work (Sissons et al., 2014) and in transgenic durum with 5 + 10 (Gadaleta et al., 2008) showed that both 2 + 12 and especially 5 + 10 addition to Svevo resulted in overly long mixograph peak time and very stable but inextensible dough (Figure 3 & Figure S1). Other authors observed a similar behavior in transgenic plants overexpressing the subunit 5 in the durum wheat cultivars Ofanto and L35 (He et al., 1999). In particular, the overexpression of the subunit 5 in L35 led to the production of doughs too strong for conventional mixograph analysis, resulting in erroneously low mixing time and peak resistance. Removal of the 7 + 8 in Svevo with 5 + 10 and 2 + 12 resulted in dough properties more like Svevo for mixograph (Figure S1) and Force/Area (Figure 3). Interestingly, the Glutopeak peak mixing time was more sensitive to differences in dough properties with significant increases in Svevo with 2 + 12 and especially with 5 + 10 in 2014 data (Table 2, Figure 3). This suggests the Glutopeak is more sensitive to changes in dough properties as noted previously. For the lower amylose genotypes, SvLA and SvWx4A have similar strength to Svevo, while SvWx7A is the weakest from mixograph data. Adding the 5 + 10 in these genotypes gave a consistent increase in dough strength across all the dough measures with Glutopeak peak mixing time perhaps showing the greatest increase. Clear evidence for a dough strengthening effect is shown in Figure 4.

The response plots for key pasta measures across genotypes are presented in Figures 5–7. In Lira45, the 2 + 12 does not have a large effect on pasta quality and results for combined seasons versus 2014 are consistent. However, subunits 5 + 10 show larger responses across seasons but still did not reduce overall firmness significantly although firmness was lower in 2014. However, the 5 + 10 significantly lowered stickiness in 2014. For Lira42, both subunits did not significantly increase pasta firmness when correcting for protein effects (Table 3, Figure 5), while 2 + 12 in this background reduced pasta stickiness significantly. Thus, both 2 + 12 and 5 + 10 in Lira appear to benefit this variety with lower cooked pasta stickiness (Figure 5).

Previously, the inclusion of 2 + 12 or 5 + 10 in Svevo softened the pasta with minor changes in other pasta quality measures (Sissons et al., 2014; Figure 6). Removal of the 7 + 8 from Svevo reduced the impact of these subunits with now no significant reduction in pasta firmness (Tables 3 & 4, Figure 6) which is desirable. Other measures of pasta quality (stickiness and cooking loss) showed only minor changes except for some significant increases in water absorption in 2014 season for Sv5+10(A6) and Sv2+12(3) although in practice, going from 157% to 164% while statistically significant is very minor and the impact

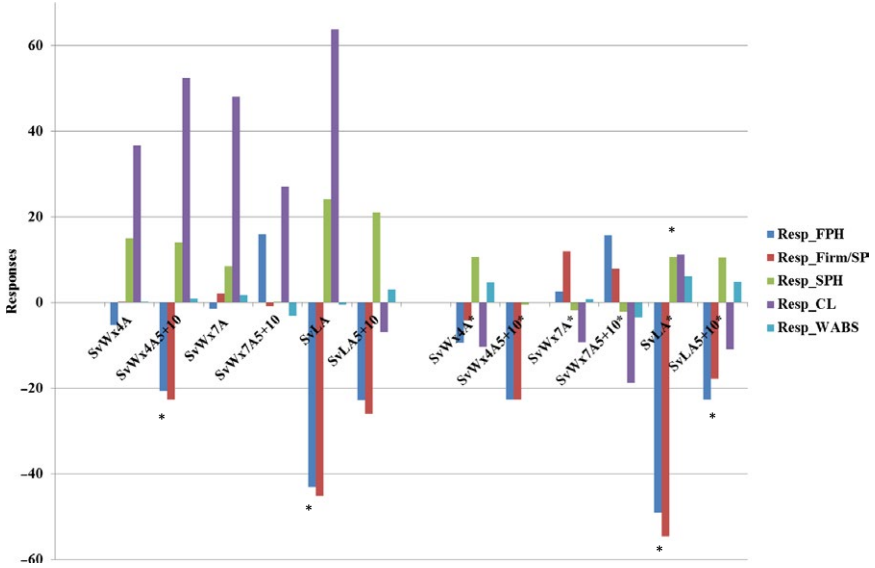

FIGURE 7 Pasta quality responses for lower amylose genotypes combined 2013/2014 and 2014 (*) seasons. Response defined as % change relative to control genotype. The * over bars shows where the means are significantly different to respective control genotypes for that set [Color figure can be viewed at wileyonlinelibrary.com]

on sensory mouthfeel is unknown. For the waxy set, there is consistent softening of SvLA pasta across seasons which tend to be stickier with higher cooking loss (Figure 7, Tables 3 & 4), while the partial waxy was similar pasta properties to Svevo. The impact of 5 + 10 in SvLA was mainly to improve firmness from 23 to 32.6 significantly (Table 4, Figure 7) but still softer than Svevo. Starch swelling is thought to be a key factor in influencing the pasta firmness in waxy durum which can swell more and lead to pasta softening (Gianibelli et al., 2005) although water absorption was not affected. Possibly, the interaction between the 5 + 10 and LMW-GS in SvLA creates a more compact pasta structure, reducing starch swelling and creating a firmer pasta. However, SvWx4A 5 + 10 pasta became softer than SvWx4A (Figure 7) differing only from SvLA 5 + 10 in their amylose content (Table 1). This was not the case for SvWx7A 5 + 10 where the 5 + 10 had no significant effect on firmness compared to SvWx7A.

3.3 | Starch properties

The paste viscosities of ground pasta were assessed using the RVA, and all the parameters except peak temperature showed significant genotype differences (Table S2). For the Lira biotypes, Lira42 showed lower peak and final viscosity (PV/FV) and breakdown than Lira45 and both types had higher values for these parameters with added 2 + 12/5 + 10 except Lira45, 5 + 10 for final viscosity. For Svevo, a similar effect of the 2 + 12/5 + 10 subunits increases peak, final viscosity, and breakdown compared to Svevo. For Svevo, the 5 + 10 had higher peak and final viscosity than 2 + 12 but this was not found in the Lira comparison. For the waxy series, SvLA had much lower peak, final viscosity, and breakdown than Svevo and the partial waxy genotypes with earlier peak time. This result is different to other reports showing higher peak viscosity in durum, barley, and maize waxy starches but alike in terms of lower final viscosity and reduced peak time compared to non-waxy (Gianibelli et al., 2005; Grant et al., 2001). The reasons for this are not clear but may be related to the SvLA sample having intermediate amylose content ~14%–15% (Table 1). Adding 5 + 10 to SvLA greatly increased viscosities. Interestingly, 5 + 10 added to SvWx4A lowered viscosities which were the opposite effect with SvWx7A and SvLA.

4 | CONCLUSIONS

Adding 2 + 12 or 5 + 10 to Lira genotypes increased dough strength assessed by several different methods but had limited effect in Svevo although the Glutopeak detected significant increases in PMT. The over strong and inextensible doughs in Svevo with 7 + 8 present, especially with 5 + 10, can be overcome by removal of the 7 + 8 giving a more balanced mixogram. The impacts on pasta were variable depending on the genotype. For Lira42, the 2 + 12 lowered stickiness and cooking loss while stickiness was only reduced in Lira45 with 5 + 10. For Svevo (without 7 + 8), there was little to no impact on pasta quality from the presence of either 2 + 12 or 5 + 10. The very low amylose Svevo (SvLA) pasta quality was improved greatly by 5 + 10 increasing firmness and reducing stickiness and cooking loss although still softer than Svevo. This approach with the aim to improve bread-making quality of durum wheat did not have an overall large negative impact on pasta-making quality and, indeed, provided some benefits to SvLA (waxy type) and Lira (weak dough type).

ACKNOWLEDGMENTS

We acknowledge the NSW Department of Primary Industries for supporting this work in particular, Denise Pleming for the Farinograph data. The research was partially supported by MIUR (Ministry for Education, University and Research), Law 232/2016, "Departments of excellence."

CONFLICT OF INTEREST

None declared.

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SUPPORTING INFORMATION

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How to cite this article: Sissons M, Sestili F, Lafiandra D. Effect of *Glu-D1* introgression on dough- and pasta-making quality of durum wheat lines with different glutenin composition and amylose content. *Cereal Chem*. 2019;00:1–13. https://doi.org/10.1002/cche.10137