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RESEARCH ARTICLE

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Effect of *Glu-D1 gene* introgression and amylose content on breadmaking potential of blends of durum and hexaploid wheat

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Abstract

Background and objectives: Durum wheat is used to make leavened bread; however, durum bread has inferior loaf volume, structure, and texture compared to bread made from common wheat. One approach to overcome this is to transfer key storage protein genes present at the *Glu-D1* locus from bread wheat into durum. Durum wheat Svevo missing *Glu-B1* subunits 7 + 8 and Lira biotypes with low molecular weight glutenin subunits types 1 and 2 were evaluated for their breadmaking potential with and without high molecular weight glutenin subunits 2 + 12 or 5 + 10.

Findings: Bread made from blends of durum and a commercial baker's flour (10%, 25%, 50% w/w) assessed over two seasons in 10 different genotypes showed that as more durum was included in the mixture, loaf volume and texture score declined. Incorporation of the 2 + 12 subunit pair in the genotypes Lira42 and Lira45 improved bread quality but not in Svevo, whereas including 5 + 10 improved bread quality of Lira42 had no effect on Lira45 but reduced quality of Svevo. Low amylose Svevo had similar loaf quality to Svevo while adding 5 + 10 had minimal impact except at 50% with a small improvement in loaf quality. Bread stored up to 7 days became firmer partly due to increased starch retrogradation, and loaves were similar to bread made from baker's flour. Low amylose Svevo kept the loaf fresher but only up to 3 days of storage. Subunit pair 5 + 10 made the loaf firmer after 7 days compared to control.

Conclusions: Addition of the 2 + 12 or 5 + 10 benefited the weaker type gluten as found in Lira 42 (LMW-1, HMW 20) but not with stronger dough in Svevo even in the absence of HMW 7 + 8. It appears that while *Glu-D1* subunits are critical for good breadmaking in hexaploid wheat, they appear to have limited value in improving loaf volume and structure in durum bread.

Significance and novelty: Some improvements in bread quality can be obtained by introducing genes coding for *Glu-D1* subunits 2 + 12 and 5 + 10 in durum wheat depending on the genotype, especially weak dough types, and the results presented comprise the first report of such effect.

KEYWORDS
durum wheat, gliadin, gluten strength, glutenin, leavened bread quality

1 | INTRODUCTION

Durum wheat (*Triticum turgidum* L. var durum) has many uses as a food crop, and though mostly used in pasta production, there is a growing interest in its use for making bread with about 25% of the durum wheat produced in the world used for breadmaking and up to 70%–90% in some Middle East countries (Pasqualone, 2012; Quaglia, 1988). Durum has inferior loaf volume, structure, and texture compared to common wheat (Boggini, Tusa, & Pogna, 1995; Guzman et al., 2016; Hareland & Puhr, 1999; Peña, Zarco-Hernandez, Amaya-Celis, & Mujeeb-Kazi, 1994) although it is preferred by some for its peculiar and distinctive sensory properties, different from common wheat bread and having a better shelf life (Pasqualone, 2012). Increasing protein content can improve loaf volume, but the inelastic and poorly extensible gluten in durum (Ammar, Kronstadm, & Morris, 2000; Boyacioglou & D'Appolonia, 1994) prevents full gas expansion, as dough extensibility is an important trait to obtain good loaf volume (Nash et al., 2006). Other characteristics (particle size, damaged starch, water absorption, protein quality, and quantity) can influence baking performance of durum (Abecassis, Cuq, Boggini, & Namoune, 2012). Wheat seed storage proteins play an important role in the manufacture of quality bread and pasta made from durum wheat. The proteins of importance that affect dough properties of flours are gliadins (monomeric proteins), responsible for dough viscosity; and glutenins (composed of high and low molecular weight glutenin subunits which form a large polymer held together by disulfide bonds when formed into a dough), responsible for elasticity and strength (Payne, 1987; Shewry, Halford, Belton, & Tatham, 2002). In bread wheat, HMW-GS determine the majority of the breadmaking quality of a flour especially those present at the *Glu-D1* locus, key subunits being 1Dx5 + 1Dy10 (5 + 10) (Li et al., 2015; Payne, 1987; Shewry et al., 2002). The HMW-GS help to form strong doughs important in baking to trap small bubbles of carbon dioxide gas formed by yeast during proofing, thereby enabling the dough to rise and give a good loaf volume and structure to leavened breads. However, durum wheat which is a tetraploid (AABB) lacks these key subunits. The maximum possible number of HMW-GS in durum wheat is three (Shewry & Halford, 2002), and this limitation and absence of D genome restricts breadmaking quality of durum. Transferring storage protein genes, present on chromosome 1D, into durum has been attempted to try to improve the breadmaking quality. However, for good pasta quality, it has been shown that the most important genes are those associated at the *Glu-B3* locus, which encodes the LMW-GS (Payne, Jackson, & Holt, 1984; Pogna, Autran, Mellini, Lafiandra, & Feillet, 1990). Improving breadmaking quality while maintaining pasta making quality is a worthwhile goal to widen the uses of durum wheat.

There are two approaches that can be used to introduce the *Glu-D1* subunits. Transgenic lines expressing additional HMW-GS genes have been reported mostly involving adding 5 + 10 to improve dough strength with the hope of improving breadmaking quality in bread and durum wheat (Barro et al., 1997; Blechl et al., 2007; Butow, Tatham, Savage, Gilbert, & Shewry, 2007; Graybosch, Seabourn, Chen, & Blechl, 2011; He et al., 1999). These works showed increases in polymeric protein, mixing times and tolerances but often produced overly strong doughs. Only the work of Graybosch et al. (2011) showed the negative impact on bread loaf volume from too much overexpression of 5 + 10. An alternative to transgenesis is chromosome engineering, a methodology capable of promoting homoeologous recombination using ph1 mutants of durum and bread wheat. Using this approach, chromosomal segments carrying the *Glu-D1* loci containing genes encoding the pairs 5 + 10 or 1Dx2 + 1Dy12 (2 + 12) have been transferred in durum wheat, replacing the null allele present at the *Glu-A1* locus on the long arm of the 1A chromosome using translocation lines 1AS.1AL.1DL in an attempt to improve durum breadmaking quality (Joppa, Klindworth, & Hareland, 1998; Klindworth et al., 2014; Lukaszewski, 2003; Sissons, Pleming, Margiotta, D'Egidio, & Lafiandra, 2014; Vitellozzi, Ciaffi, Dominici, & Ceoloni, 1997).

Another approach to modifying the properties of the durum is to add 5 + 10 and 2 + 12 to partial waxy durum. The full waxy character is detrimental to good *al dente* pasta firmness, softening the pasta (Gianibelli, Sissons, & Batey, 2005; Grant, Doehlert, McMullen, & Vignaux, 2004; Vignaux et al., 2005) while the partial waxy have much less impact (Sharma, Sissons, Rathjen, & Jenner, 2002). Bhattacharya, Erazo-Castrejón, Doehlert, and McMullen (2002) found addition of waxy durum flour to a bread flour at 20% reduced staling more effectively than the use of shortening. Furthermore, waxy durum flour has been shown to soften loaves and can partially substitute for fat in the formulation reducing fat content and cost of bread manufacture (Mouliney, Lavery, Sharma, & Jenner, 2011). Therefore, bread quality and/or staling of bread made from durum wheat with low amylose content could be improved. It is possible the lowering of amylose in combination with *Glu-D1* subunits may provide a better balance that improves bread loaf volume and quality and still retain suitable pasta making potential (Sissons et al., 2019). There is no research investigating the interaction of the waxy alleles with *Glu-D1* subunits in durum wheat.

The objectives of this work were to determine the effect on dough and leavened breadmaking quality of the addition of *Glu-D1* high molecular weight glutenin subunits 2 + 12 or 5 + 10 in the durum wheat cultivar Svevo missing the *Glu-B1* subunits 7 + 8. In addition, two Lira biotypes 45 and 42 having strong and weak LMW-GS, LMW-2 and LMW-1, respectively, and had *Glu-D1* subunits 5 + 10 and 2 + 12 were used. Svevo with low amylose and HMW-GS 5 + 10 were examined to assess their combined effects on quality characteristics.

2 | MATERIALS AND METHODS

2.1 | Plant material

Svevo with subunits 7 + 8 and 5 + 10 or 2 + 12 are described in Sissons et al. (2014). The lines of Svevo 7 + 8/5 + 10 or 7 + 8/2 + 12 were crossed with a *T. dicoccoides* line completely null for HMW-GS. In this way, two lines were produced carrying the pair of subunits 5 + 10 or 2 + 12 alone without the subunits 7 + 8. These lines were backcrossed three times with Svevo 7 + 8/5 + 10 and 7 + 8/2 + 12, respectively, and designated as Sv 2 + 12 (with no 7 + 8) and Sv 5 + 10 (with no 7 + 8). The two biotypes 42 and 45, present in the durum wheat cultivar Lira, with HMW-GS 20 were crossed with the durum wheat line WB 881 (Lukaszewski, 2003) carrying the translocations containing the genes for the subunits 5 + 10 or 2 + 12. After three backcrosses, four lines were isolated with the combinations of the biotype 45 with subunits 5 + 10 or 2 + 12 and the same for the biotype 42 (referred to as Lira42 2 + 12, Lira42 5 + 10, Lira45 2 + 12, Lira45 5 + 10). Lines of a partial waxy (genotype lacking starch granule-bound protein Wx-A1, 24.1% amylose or Wx-B1, 27.6% amylose) or a low amylose line (14.8% amylose) isolated in the durum wheat cultivar Svevo with 29.3% amylose (Lafiandra et al., 2010) were crossed and backcrossed three times with Svevo 7 + 8/5 + 10 (referred to as SvLA, SvLA 5 + 10, SvWx4A, SvWx4A 5 + 10, SvWx7A, SvWx7A 5 + 10).

All the lines along with the parents were sown in 2013 and 2014 in Viterbo, Italy, in randomized triplicate plots (1.5 by 5 m) except in 2013 no samples from Lira42 were available. An amount of N fertilizer equivalent to 100 kg N/ha was applied pre-sowing and at stem elongation phase. To obtain sufficient sample for end-product testing, replicated plots were pooled for each genotype. Details of the glutenin composition of all these genotypes are described in Sissons et al. (2019).

2.2 | Flour preparation

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 milling (Buhler, Uzwil, Switzerland) with three breaking and three sizing passages. Semolina was milled into flour by re-milling in a Buhler MLU-202 laboratory test mill (Buhler AG, Switzerland). To avoid excessive grinding pressure on the stock, the semolina was lifted to the reduction side at a feed rate of 50 g/min. Reduction passages were fitted with 160-μm wire mesh, and first- and second-pass scalpers with 675- and 335-μm mesh, respectively, producing durum flours (DF) with particle size comparable to bread wheat (95% <180 μm) and starch damage of 9.8%–12% (measured using Megazyme Starch Damage Kit). DF was combined with commercial baker's flour (Perfection baker's flour, Allied Mills, Australia) to produce blends of 10%, 25%, and 50% durum for making 100-g loaves in duplicate.

2.3 | Dough testing

Dough properties of 50% blends were determined using a DoughLAB (Perten Instruments, Australia) fitted with 50-g bowl and mixing at 120 rpm (AACC Approved Method 54-70.01). Flour water absorption (FWA) at target midline peak consistency of 665 Farinograph Units (FU), time in minutes to reach midline peak dough development (DDT), time in minutes the top curve remained above midline peak consistency (Stability), and FU loss in midline peak height to 5 min past peak (Bdown 5 min) were recorded. Stability and breakdown are regarded as indicators of tolerance to mixing, with strong, tolerant doughs having long stability and low breakdown values. All samples were tested without replication.

2.4 | Breadmaking and testing

The durum flours were baked at 10%, 25%, and 50% blends according to the standard Australian straight-dough long-fermentation method (CCD Official Method 07-02, 2010) with modifications, using 100 g flour formulation with fresh compressed yeast (3%), solidified vegetable oil (2%), sugar (1%), NaCl (1%), NH_4Cl (0.1%), and ascorbic acid (50 ppm) and α-amylase (4.7 ppm) added as bread improvers. Loaves were baked in duplicate over 2 days, with a full replicate (randomized order) occurring each day, including comparison baker's flour loaves. Bake water absorption for the durum/baker's flour blends was predicted using the FWA results for the straight flours. Doughs were mixed to optimum development in a pin mixer (National Manufacturing, USA), placed in sealed containers, and fermented at 30°C. Doughs were knocked once during fermentation, at 96 min by lightly hand kneading and passing through a Universal bread molder (Mono Equipment, UK) at 5-mm roll gap and 35 mm pressure board settings and returned to sealed containers. At completion of total fermentation time of 120 min, doughs were again molded, tinned, and proofed for 60 min at 34°C and 85% RH before baking at 215°C for 24 min in a Rotel II bakery oven (Moffat, Australia). After cooling for 45 min, loaf volume was determined using pup volumeter (National Manufacturing, Nebraska, USA). The following day external loaf appearance, crumb texture (softness and resilience), and crumb cell structure and distribution were judged subjectively against the baker's flour comparison loaves. Bake scores were awarded on the basis of a maximum 20 points for volume, 10 for external appearance, and 5 for each of the crumb parameters. The 2014 loaf baking method was identical to 2013, except loaves were baked in duplicate over 4 days, with a full replicate (randomized order) occurring in each 2-day block, including comparison baker's flour loaves.

2.5 | Bread staling study

Durum flours produced from baking experiments were stored in double-layer sealed plastic bags at 4°C until required. Three samples were analyzed from each season (Svevo, SvLA and SvLA 5 + 10) plus a commercial baker's flour control (Perfection baker's flour, Allied Mills, Australia) in the one experiment. DF was mixed with baker's flour to produce 25:75 blends. Baking was conducted using the Australian standard rapid baking method (CCD Official Method 07-03, 2010) with slight modifications including omission of acetic acid. Formulation included 2% salt, 1% fat, 0.025% NH_4CL, 60 ppm ascorbic acid, 5.5 ppm α-amylase, and 3% compressed yeast. Bake water addition was calculated based on the water absorption obtained for 50% DF blends using a DoughLAB. At baking, replicate doughs were produced for each sample in a DoughLAB fitted with 300-g bowl, mixing at 180 rpm to just past development peak. Doughs were rested for 5 min after completion of mixing, scaled into three 150 g pieces, passed through Mini Molder (Mono Equipment, UK) at 5-mm gap and 35 mm pressure board settings, placed in sealed containers, and rested 10 min at 30°C. Doughs were passed through Mini Molder before placing in bake tins and proofing for 70 min at 85% RH and 34°C. Loaves were baked for 20 min at 214°C in a Rotel II bakery oven (Moffat, Australia), and then cooled 40 min before weighing and volume determination using a pup volumeter. After a further 80-min cooling, two central 20-mm-thick slices were removed from each loaf, trimmed of crust to minimize moisture movement, immediately heat-sealed in laminated PET MET/LDPE pouches (Flexpak, Australia), and stored at room temperature in sealed plastic containers until required for texture analysis.

2.5.1 | Texture analysis

Three slices from each sample were randomly assigned to each of three storage treatments: 1, 3, and 7 days after removal from oven (a further treatment of 10 days of storage was abandoned due to sample deterioration). After removal from the pouch, slices were presented to a TA-XT2 analyzer (Stable Micro Systems, UK) fitted with a 45-mm flat-based cylindrical probe, using standard TPA ("2 bite") compression test (speed 1 mm/s, compression distance 40% of slice height, pause 3 s). Both sides of the slice were tested yielding six results per sample per storage treatment. Immediately following texture analysis of each slice, a 20 × 20 mm section underwent moisture analysis using the two-stage bread moisture method (AACC Method 44-15.02) utilizing a MA37 moisture analyzer (Sartorius Instruments, Germany). Crumb firmness was defined as the peak force attained in the first compression. Springiness was the ratio of the distance under the second compression curve to peak force (D2) to the distance of the first compression peak force (D1) and is indicative of how well the crumb physically springs back after being deformed and rested. Resilience was the ratio of the area of work on the upstroke to the area of work on the downstroke of the first compression and indicates the crumb resistance (or "fight back") to deformation. Cohesiveness was the ratio of the area of work of the second compression to the area of work of the first compression, indicating the ability of the crumb to withstand a second deformation relative to its resistance under the first. Gumminess and chewiness were defined as firmness X cohesiveness and gumminess X springiness, respectively.

2.5.2 | Differential scanning calorimetry

At the time of texture analysis, a small section (10 × 10 mm) was removed from each slice and stored at −20°C in sealed plastic bags before freeze drying and storing in sealed vials. A small section was then hand crushed and weighed (approximately 20 mg, wetted with approximately 40 mg distilled water) into sealed 100-µl aluminium crucibles. Samples were scanned using a DSC822 (Mettler Toledo, USA) calibrated with indium, at a scan rate of 10°C/min from 20 to 95°C. Parameters obtained were enthalpy, onset, peak, and endset gelatinization temperature.

2.6 | Statistical methods

Composite samples (from the field replicates) were prepared for breadmaking to ensure sufficient sample and reduce the number of analyses and baked in duplicate. For 2014, the full set of genotypes were available and analyzed using bake replicate as a block with a balanced one-way analysis of variance (ANOVA) using Statistical Analysis System (GenStat 11.1, VSN International Ltd.) software. For genotypes in common between the years, mean values across all the DF levels were calculated and analyzed by ANOVA to determine genotype and year effects. Data are presented as means and compared for significant differences ($p < 0.05$) using the least significant difference statistic (LSD) where appropriate.

3 | RESULTS AND DISCUSSION

3.1 | Comparison of the effect of *Glu-D1* subunit addition on breadmaking properties across seasons and genotype backgrounds

3.1.1 | 2014 season results

Based on previous work incorporating durum flour (DF) from these introgressions (Sissons et al., 2014) with baker's premium flour (BF) as a base for breadmaking, we only made bread from 10%, 25%, and 50% DF:BF combinations

as higher incorporations resulted in excessively long bakery mix times and very poor loaf characteristics.

Mean baking data for 15 genotypes are shown in Table 1 with comparisons made using superscript letters according to genotype group (Lira, Svevo types). The Lira42 series were available for this set representing the LMW-1 compared to the LMW-2 types in Lira45, the former known for making weaker dough (Pogna et al., 1990), which would explain the much shorter bake mix time. This was evident in the fast Farinograph data showing longer DDT, stability, and lower breakdown in Lira45 compared to the Lira42 genotypes (Supporting information Table S1). Generally, as more DF was used in the bake mix, especially going from 25% to 50%, mix times increased beyond the BF mix of 195 s. A similar behavior was reported in transgenic bread wheat lines overexpressing HMW-GS subunits: The effect was less drastic for the subunit 1Ax1 and 1Dy10 than that described for the 1Dx5 subunit. In the last case, extra strong mixing characteristics were described (Rakszegi et al., 2005, 2008; Blechl et al., 2007; Field et al., 2008; Wang et al., 2010; Graybosch et al., 2011). For the Lira set, adding 2 + 12 only increased mix time at DF 25% or 50% compared to Lira control. While adding 5 + 10 to Lira42 increased mix time from DF 10% but only from 25% for Lira45. The 5 + 10 gave longer mix times compared to 2 + 12 at 50% DF. The Svevo 2 + 12 at all DF levels had similar mix times to Svevo (although fast Farinograph showed a more stable dough, Supporting information Table S1) while the Svevo with 5 + 10 increased the mix time at 25 and 50% DF relative to Svevo and this was more than double mix time for BF. Compared to Svevo, the SvLA mix times were similar while SvWx4A was slightly lower and SvWx7A was lower than SvWx4A at 25 and 50%DF. The addition of 5 + 10 in all waxy types increased mix time significantly at 10% (but not for SvLA), 25% and 50% DF, with a very long mix time for SvWx7A 5 + 10 of 473 s. These data are consistent with the effects seen in dough tests where addition of these subunits increased dough strength (Sissons, Sestili & Lafiandra, 2019) and as shown by fast Farinograph data showing more stable and stronger dough with 5 + 10 presence (Supporting information Table S1). All the samples had a higher Farinograph water absorption than baker's flour (at 50% DF) especially in the waxy series with the SvLA having the highest as lower amylose durum flour has a higher water absorption (Gianibelli et al., 2005) (Supporting information Table S1). However, these differences had minimal impact on bake water absorption as calculated bake water absorption (BWA) was limited to a maximum of 66.5% (equivalent to FWA of 68.5) in order to avoid dough handling problems resulting from excessive water addition.

The highest LV was obtained with BF (975cc) while there were several lines with similar LV using 10% DF (Figure 1, Table 1). As the amount of DF increased in the bake mix, LV decreased showing the impact of durum flour and 50% DF depressed LV relative to BF approximately 27%–32%. Total loaf score was lower than BF (48.3) in the durum loaves at all DF rates, except for Lira45 at 10% DF and tended to also decrease with increase in DF. For the Lira series, Lira45 gave marginally higher LV compared to Lira42 but was only significantly higher at 50% DF. Total loaf score was also higher for Lira45 at 10 and 50% DF. Adding the 2 + 12 or 5 + 10 to Lira42 increased LV and total score at only 25 and 50% DF. However, in Lira45 only 2 + 12 at 50% DF significantly increased LV and total score. This could be related to the stronger dough of Lira45 having the LMW-2 subunits compared to Lira42 with LMW-1 and having a weaker dough (Sissons et al., 2019). In the Svevo background, the presence of 2 + 12 did not significantly change LV and TS but 5 + 10 depressed LV at all DF levels with no reduction in total score until 50% Sv 5 + 10. In contrast, earlier work showed that 2 + 12 in the presence of 7 + 8 in Svevo depressed LV but not TS while the 5 + 10 had an even larger deleterious impact on LV (Sissons et al., 2014). This again shows the effect of the *Glu-D1* subunits on baking performance varies with the genetic background. Possibly when 2 + 12 is present with 7 + 8, the greater total number of HMW-GS would lead to more cross-links, strengthening the dough and limiting loaf volume expansion. Dhaka and Khatkar (2014) found a strong positive relationship between HMW-GS/LMW-GS ratio and specific loaf volume. Possibly the balance between elasticity and viscosity when both subunit pairs are present is less than ideal for optimum loaf volume.

In the waxy series, LV and total score were similar to Svevo with inconsistent effects at the different DF levels. The impact of adding the 5 + 10 to each of these three waxy types on LV was variable but showed significant increases at 50% DF for the two partial waxy. All loaves produced bright crumb with high L^* values ~80–82, and 50% DF loaves were slightly more yellow b^*~16–19 (data not shown).

Increasing amounts of DF resulted in a decline in loaf external appearance and crumb texture. Impact on crumb characteristics was most obvious in the crumb structure scores, declining markedly as DF inclusion rates increased to 50%. Crumb structure for Lira42 was better maintained at 50% DF with 2 + 12 present and at all DF levels with 5 + 10. These benefits in crumb structure were not apparent in Lira45 and Svevo, and this was the case for the other loaf parameters (Table 1). Compared to Svevo, generally the cell distribution and cell structure scores for the SvLA, SvWx4A, and SvWx7A were inferior. The presence of the 5 + 10 in these samples had variable and mostly no significant changes in these samples.

3.1.2 | Combining both seasons' data

Mean values across all DF levels for each genotype according to season were calculated and subject to ANOVA to determine year effect. Significant year effects were found for LV, loaf

TABLE 1 Breadmaking data on genotypes and blends of durum flour with commercial baker's flour, 2014 season

% Durum flour	Genotype	Mix time (s)	BWA%	Loaf external (of 10)	Loaf Vol (cc)	Vol score (vol/48) (of 20)	Crumb softness (of 5)	Crumb resilience (of 5)	Crumb structure (of 5)	Cell distribution (of 5)	Total loaf score (of 50)
0	Bakers	195	64.9	9.0	975	20.3	5.0	4.5	5.0	4.5	48.3
10	Lira42	165^{a}	63.8	7.5	913^{ab}	19.0	4.0	4.5	4.5	4.0	43.5^{a}
10	Lira42 2 + 12	210^{bce}	63.9	8.5	940^{a}	19.6	5.0	4.0	4.5	4.0	45.6^{bc}
10	Lira42 5 + 10	200^{b}	63.5	8.0	888^{bc}	18.5	5.0	4.0	5.0	4.0	44.5^{ab}
10	Lira45	203^{bc}	64.1	8.5	935^{a}	19.5	5.0	5.0	5.0	4.0	47.0^{c}
10	Lira45 2 + 12	205^{bc}	64.2	8.0	870^{ce}	18.1	4.0	4.5	4.0	4.5	43.1^{ae}
10	Lira45 5 + 10	210^{bce}	64.0	8.0	900^{b}	18.8	5.0	4.0	5.0	3.5	44.3^{ab}
25	Lira42	203^{bc}	63.8	7.5	805^{d}	16.8	4.0	4.5	3.5	3.5	39.8^{d}
25	Lira42 2 + 12	193^{c}	64.0	8.0	845^{ef}	17.6	4.5	4.5	3.0	3.0	40.6^{de}
25	Lira42 5 + 10	225^{e}	63.0	8.0	845^{ef}	17.6	4.5	4.0	4.0	3.5	41.6^{e}
25	Lira45	210^{bce}	64.3	7.0	828^{edf}	17.2	4.5	4.5	3.5	4.0	40.7^{de}
25	Lira45 2 + 12	250^{g}	64.7	7.5	855^{ef}	17.8	5.0	4.5	4.5	4.5	43.8^{a}
25	Lira45 5 + 10	263^{g}	64.2	7.0	838^{f}	17.5	4.5	4.5	4.5	3.5	41.5^{e}
50	Lira42	218^{be}	63.6	6.5	663^{g}	13.8	3.0	3.5	1.0	1.0	28.8^{f}
50	Lira42 2 + 12	270^{d}	64.0	6.5	730^{hi}	15.2	4.5	4.0	2.5	4.0	36.7^{g}
50	Lira42 5 + 10	293^{f}	62.0	6.5	743^{h}	15.5	3.5	4.5	2.5	4.0	36.5^{g}
50	Lira45	248^{g}	64.7	6.5	700^{i}	14.6	3.5	4.0	1.5	1.5	31.6^{h}
50	Lira45 2 + 12	320^{h}	65.5	8.0	748^{h}	15.6	3.5	4.0	2.0	3.0	36.1^{g}
50	Lira45 5 + 10	355^{i}	64.5	5.0	708^{i}	14.7	4.0	4.0	1.0	1.5	30.2^{f}
10	Svevo	208^{A}	64.3	8.0	913^{AC}	19.1	5.0	4.5	4.5	4.0	45.1^{AF}
10	Sv 2 + 12(A3)	208^{A}	64.1	8.0	895^{AB}	18.6	5.0	4.5	5.0	4.5	45.6^{A}
10	Sv 5 + 10(A4)	200^{A}	64.2	9.0	880^{BF}	18.4	5.0	3.5	5.0	3.5	44.4^{AF}
25	Svevo	228^{B}	65.0	7.0	865^{D}	18.0	5.0	4.5	4.0	3.5	42.0^{BEF}
25	Sv 2 + 12(A3)	225^{BG}	64.4	8.0	840^{DEF}	17.5	4.5	3.5	3.0	4.0	40.5^{B}
25	Sv 5 + 10(A4)	260^{E}	64.7	7.5	825^{E}	17.2	4.5	4.0	4.5	3.5	41.2^{BE}
50	Svevo	288^{C}	66.0	6.5	698^{HKJ}	14.5	3.5	4.0	2.0	2.5	33.0^{C}
50	Sv 2 + 12(A3)	295^{C}	64.9	6.5	688^{HK}	14.3	3.5	3.5	1.5	2.5	31.8^{CH}
50	Sv 5 + 10(A4)	410^{F}	65.5	6.0	620^{I}	12.9	3.0	3.0	1.0	1.0	26.9^{D}
10	SvLA	193^{A}	64.9	7.5	883^{BF}	18.4	5.0	3.0	4.5	3.0	41.4^{B}

(Continues)

TABLE 1 (Continued)

% Durum flour	Genotype	Mix time (s)	BWA%	Loaf external (of 10)	Loaf Vol (cc)	Vol score (vol/48) (of 20)	Crumb softness (of 5)	Crumb resilience (of 5)	Crumb structure (of 5)	Cell distribution (of 5)	Total loaf score (of 50)
10	SvLA 5 + 10	203^{A}	64.2	8.0	925^{C}	19.3	5.0	4.0	5.0	4.0	45.3^{A}
10	SvWx4A	178^{D}	64.5	8.0	875^{B}	18.2	5.0	4.0	4.0	3.0	42.2^{EF}
10	SvWx4A 5 + 10	210^{AG}	64.3	8.5	920^{AC}	19.2	5.0	4.0	4.0	3.0	43.7^{F}
10	SvWx7A	188^{D}	64.6	9.0	900^{ABC}	18.8	5.0	3.0	4.0	3.5	43.3^{F}
10	SvWx7A 5 + 10	203^{A}	64.3	8.5	913^{AC}	19.1	5.0	4.5	4.5	4.0	45.6^{A}
25	SvLA	200^{A}	66.3	8.0	873^{BFG}	18.2	4.5	2.0	2.5	2.5	37.7^{G}
25	SvLA 5 + 10	220^{G}	64.6	7.5	863^{F}	18.0	4.5	2.0	3.5	3.0	38.5^{G}
25	SvWx4A	208^{A}	65.4	7.5	848^{DEG}	17.7	4.5	3.5	4.0	4.0	41.2^{BE}
25	SvWx4A 5 + 10	258^{E}	64.9	8.0	820^{E}	17.1	4.5	3.0	3.0	3.0	38.6^{G}
25	SvWx7A	185^{D}	65.7	8.0	833^{E}	17.4	5.0	4.0	3.0	3.5	40.9^{B}
25	SvWx7A 5 + 10	288^{C}	64.9	8.0	840^{DE}	17.5	4.0	4.0	3.0	3.5	40.0^{B}
50	SvLA	208^{A}	66.5	6.5	713^{HJ}	14.9	4.5	1.0	1.5	2.0	30.4^{H}
50	SvLA 5 + 10	280^{C}	65.4	7.0	740^{J}	15.4	4.0	2.0	2.0	1.0	31.4^{H}
50	SvWx4A	255^{E}	66.5	6.5	673^{K}	14.1	4.0	3.5	2.0	2.0	32.1^{CH}
50	SvWx4A 5 + 10	418^{F}	65.9	7.0	725^{J}	15.1	3.5	2.0	1.5	2.0	31.1^{H}
50	SvWx7A	218^{BG}	66.5	5.5	640^{L}	13.3	3.0	3.0	1.0	1.5	27.3^{I}
50	SvWx7A 5 + 10	473^{H}	65.8	6.0	700^{HJ}	14.6	3.5	3.0	2.0	2.0	31.1^{H}
	LSD Gx%flour	17.9		0.74	26.7	0.6	0.5	0.6	0.5	0.6	1.5
	Gx%flour	<0.001		0.1	0.008	0.007	0.2	0.07	<0.001	0.004	<0.001

Note. Numbers with alike letters in the same column and same group (Lira or Svevo type) are not statistically different, $p < 0.05$. Comparisons made against control genotypes within groups (Lira42/45, Svevo).

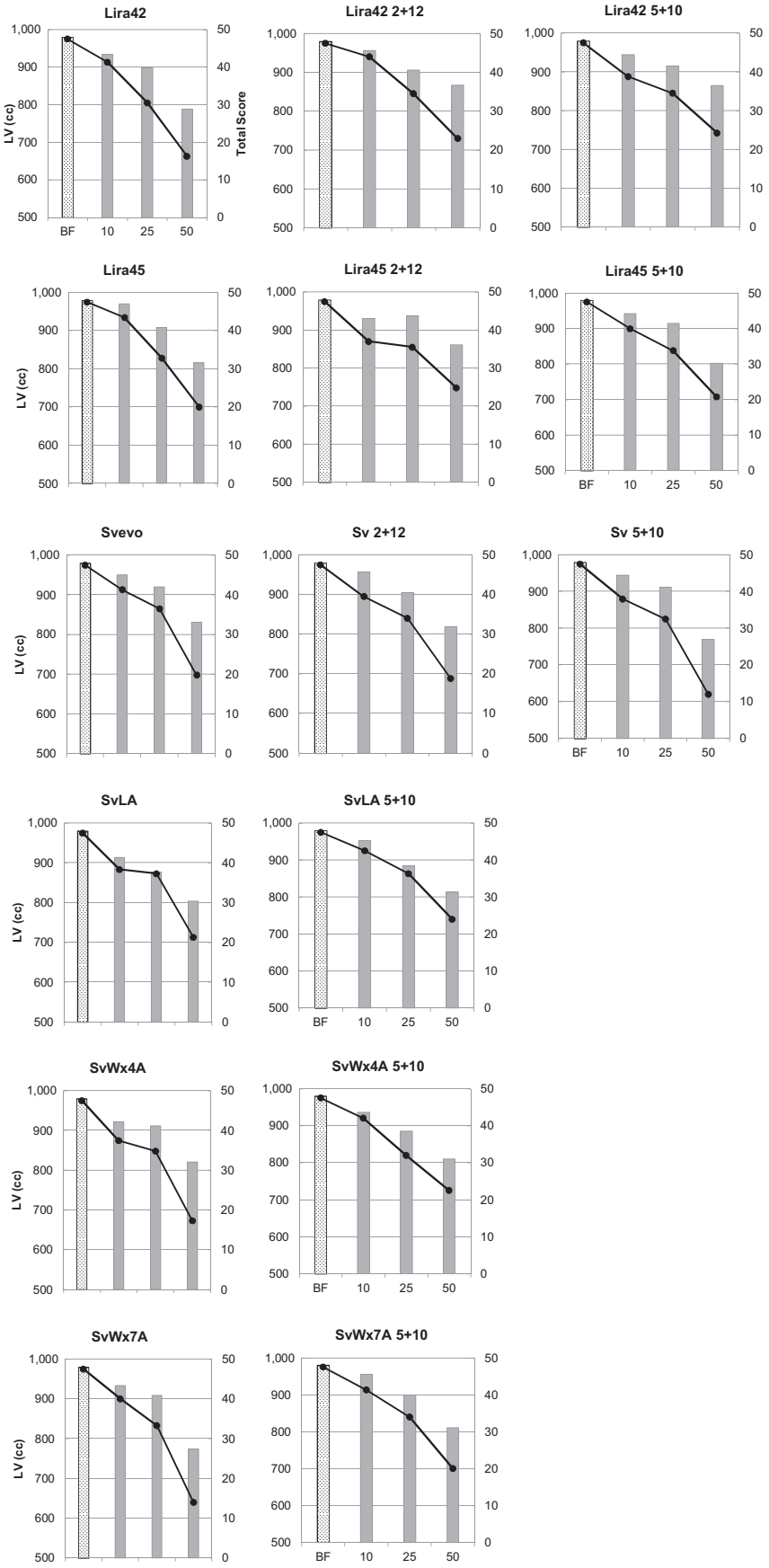

FIGURE 1 Loaf volume (line) and total score (bar) of breads made from blends of durum flour and baker's flour at 10%, 25%, and 50%. Data are means from 2014 season with LSD for LV = 27 and TS = 1.5

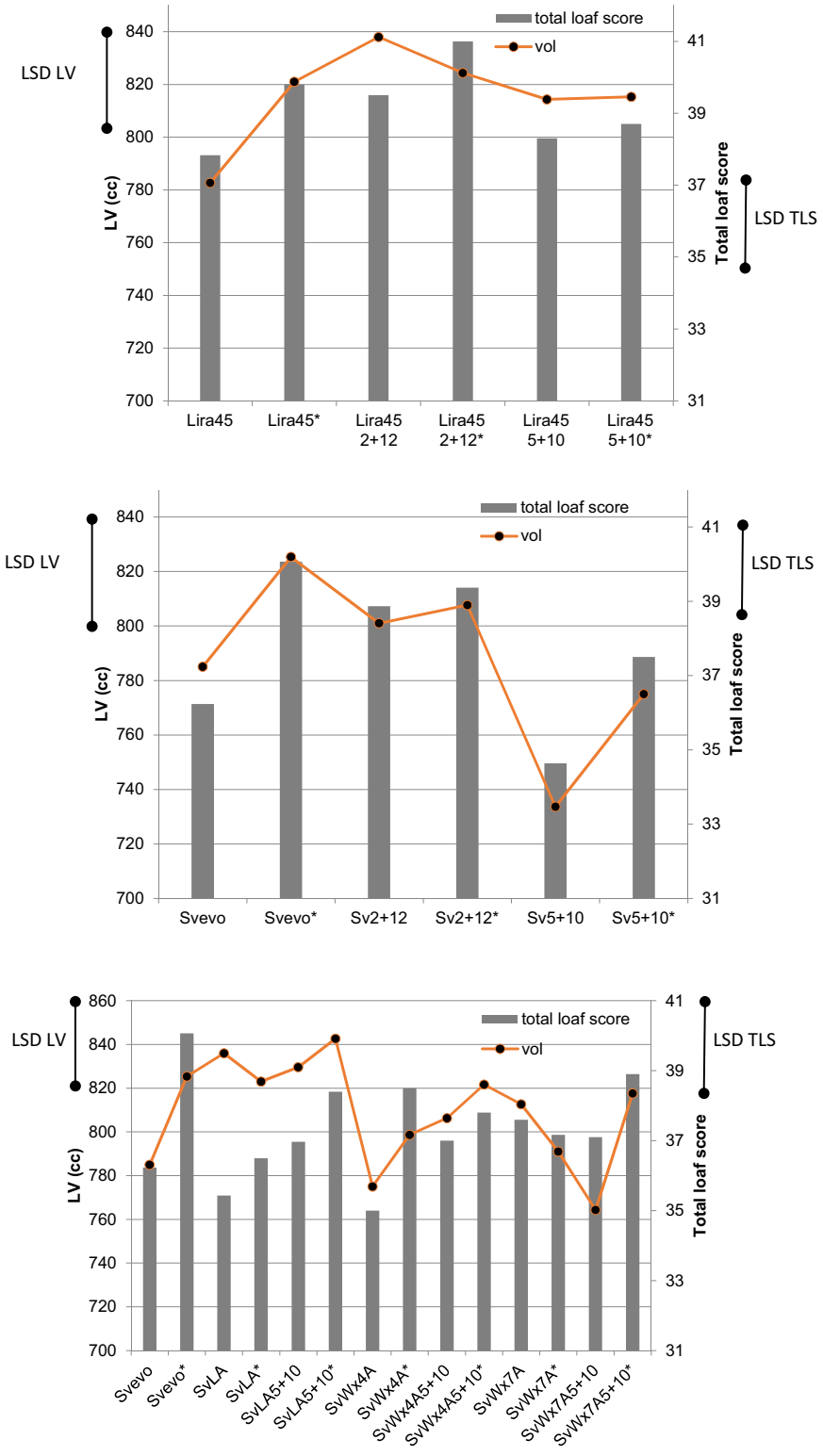

FIGURE 2 Mean loaf volume (line) and total loaf score (bar) of breads for genotypes according to season. Data are means across all durum flour %. An * after genotype name reflects the 2014 season. LSD for LV = 40 and TS = 2.6

external, volume score, total loaf score, and crumb structure (data not shown). These data are summarized in Figure 2 divided into genotype group. The 2 + 12 inclusion only increased LV in 2013 sample, and 5 + 10 had no significant affect with no loaf score effects from either subunit pair. This is consistent with the LV responses (% change) relative to BF loaf (Supporting information Figure S1). However, for Lira42 at 25% and 50% DF, slight improvements in LV were achieved with presence of 2 + 12 and 5 + 10 for the only season it was trialed (2014) (Figure 1). In Svevo, the 2 + 12 did not significantly change LV and TS while 5 + 10 depressed LV in both seasons at 50% DF (Figure 2). SvLA had significantly higher LV than Svevo in 2013 but not 2014 while both SvWx4A and SvWx7A across both seasons had similar LV to Svevo. Inclusion of 5 + 10 had

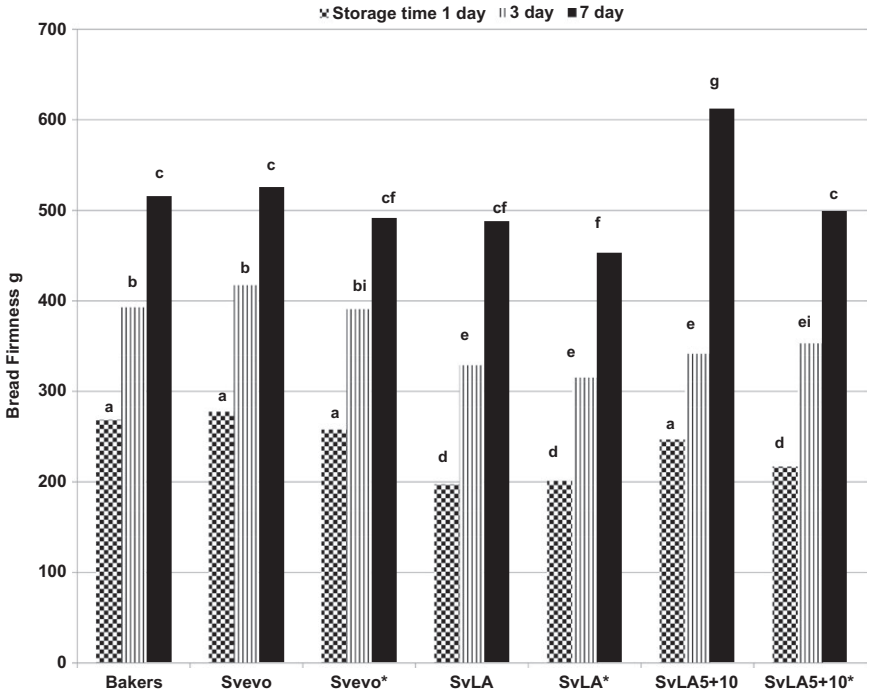

FIGURE 3 Effect of storage time on durum flour–baker's flour (25:75) mixes for the genotypes on bread firmness. Lines grown in 2014 season indicated with *. Alike lowercase letters above bars are not significant, $p < 0.05$

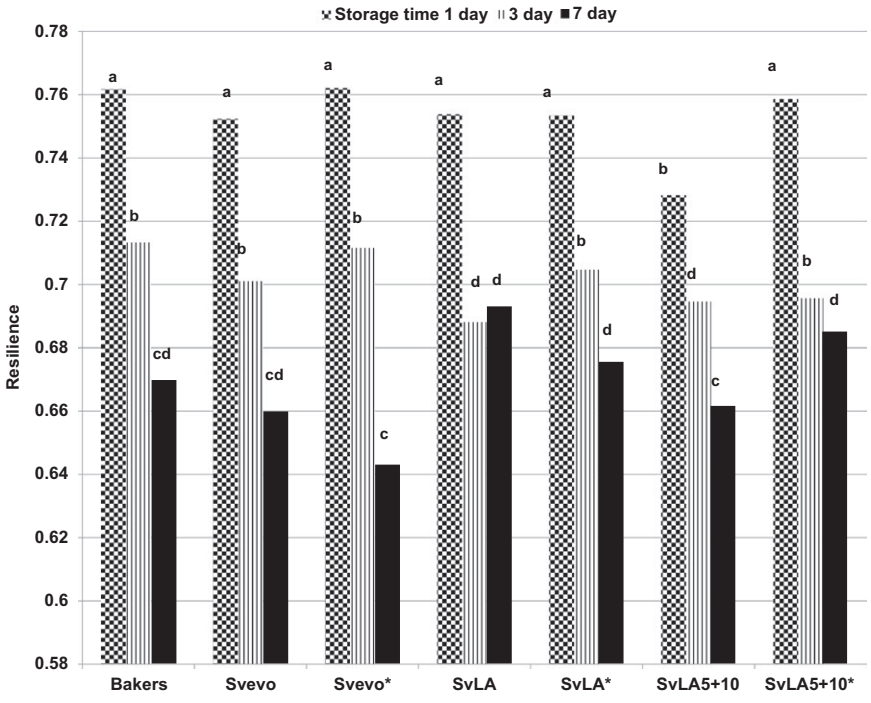

FIGURE 4 Effect of storage time on durum flour–baker's flour (25:75) mixes for the genotypes on bread resilience. Lines grown in 2014 season indicated with *. Alike lowercase letters above bars are not significant, $p < 0.05$

no significant effect on LV in SvLA, SvWx4A, and SvWx7A (except it was lower in 2013 for SvWx7A).

Overall, the closest LV and TS to baker's loaf was achieved using only 10% DF in the mix. This agrees with the findings of researchers working across a diverse range of products such as lupin flour (Correia, Gonzaga, Batista, Beirão-Costa, & Guiné, 2015), chickpea flour (Mohammeda, Ahmeda & Senge, 2011), oat flour (Majzoobi, Jalali, & Farahnaky., 2016), and grape pomace (Walker, Tseng, Cavender, Ross, & Zhao, 2014) who have all found that optimum rates of substitution are 10% or less if acceptable loaf volume and crumb character is to be maintained. Guzman et al. (2016) noted several durum varieties produced LV similar to bread wheats in some environments, particularly under drought stress. Previous work showed that 2 + 12 and 5 + 10 added to Svevo reduced LV (Sissons et al., 2014). In the present

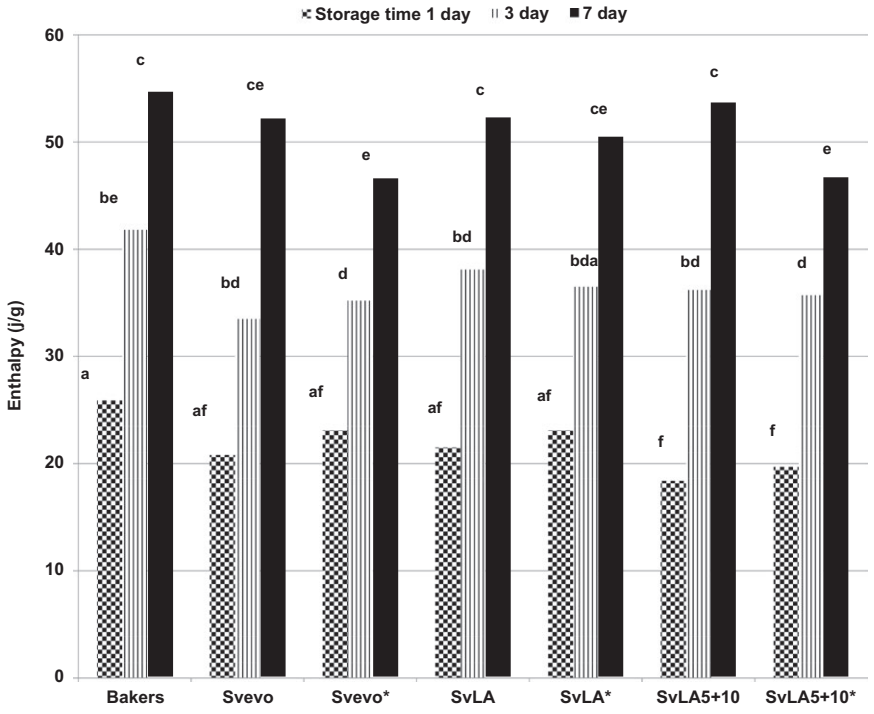

FIGURE 5 Effect of storage time on durum flour–baker's flour (25:75) mixes for the genotypes on differential scanning calorimetry enthalpy values. Lines grown in 2014 season indicated with *. Alike lowercase letters above bars are not significant, $p < 0.05$

study, removal of the 7 + 8 in Svevo resulted in a more balanced and less inelastic dough mixogram (see Sissons et al., 2019), and this translated into a less negative impact from 5 + 10 on the LV and now, the inclusion of 2 + 12 no longer reduced LV of Svevo, but also did not improve it. Clearly, the balance of glutenin subunits is still not ideal possibly because what is needed is more extensibility in the dough. The 2 + 12 subunit pair was more effective in Lira at 25 and 50% DF inclusion possibly because Lira has *Glu-B1* HMW-GS 20, which presents less strength to the dough than 7 + 8 present in Svevo (Ammar et al., 2000; Brites & Carillo, 2001; Sissons, Ames, Hare, & Clarke, 2005) which might allow for more gas expansion during bread baking. However, Ammar et al. (2000) noted that durum carrying the HMW-GS 6 + 8 produced bread loaves that were larger than those produced by genotypes having the 7 + 8 or 20 probably due to their higher dough extensibility. These researchers concluded that in order to produce durum wheat with baking performance equivalent to bread wheat, greater dough strength but, more importantly, extensibility is needed. The need for more strength in the dough is illustrated when 2 + 12 or 5 + 10 is added to the weak dough from Lira42 lifting LV at 25 and 50% DF to levels equivalent to Svevo (Figure 1).

3.2 | Effect of staling on bread

Bread staling causes a decline in consumer acceptability and typically is reflected in an increase in textural firmness and a loss of moisture and flavor. In our study, we removed the moisture loss in our experimental design as shown by crumb moisture content remaining relatively constant (42.2%–43.7%) which would otherwise affect texture in the samples if moisture declines (Supporting information Table S2). In this study, we used a maximum incorporation rate of DF of 25% as higher levels of waxy flour impacts bread structure adversely (Purna, Miller, Seib, Graybosch, & Shi, 2011 and Table 1). The effect of storage on the texture of the bread is shown in Supporting information Table S2 and for firmness and resilience in Figures 3 and 4. All samples showed a significant increase in firmness from 1 to 7 days, with bread containing 25% DF firming at the same rate as 100% BF. Genotypes from the 2013 and 2014 season showed very close correspondence in their firmness except SvLA 5 + 10 at 7 days. SvLA loaves are softer than Svevo and Baker's after 1- and 3-days storage and dent very easily under thumb pressure which is typical of waxy durum bread (Bhattacharya et al., 2002; Mouliney et al., 2011). At 7 days however, there is no difference in firmness between SvLA and Svevo. This is in contrast to Bhattacharya et al. (2002) who reported a decrease in firmness over 5 days with waxy wheat flour substituted at 40% but consistent with Purna et al. (2011) who also found no firmness difference after 7 days staling in waxy wheat bread compared to control using 15%–45% waxy wheat flour. Differences between studies may relate to different genotypes used, amylose contents of waxy types, and rates of incorporation of waxy flour interactions. The presence of the 5 + 10 in the SvLA was not consistent with a firmer loaf at days 1 and 7 in 2013 sample and only at day 7 in 2014 sample compared to respective controls. The lower firmness could be due to lower amylose content in the SvLA as suggested by Purna et al. (2011). The rate of staling (firmness increase over the 7 days) was slightly faster

for the SvLA and highest for the SvLA 5 + 10, compared to Svevo. Higher firming rates have been noted by Inagaki and Seib (1992), using cross-linked waxy barley starch which was initially softer than control but firmed much faster during further storage. Clearly, the best anti-staling was obtained with the waxy loaves but only for 3 days with a marginal benefit. Another key property, resilience, is a measure of how well a product retains original height immediately after removal of deforming pressure (Figure 4). The pattern was the reverse of firmness in that as time proceeded, loaf resilience decreased. There were no differences between Baker's and Svevo at all days and after 1 day, all samples had the same resilience (except SvLA and SvLA 5 + 10). SvLA retained a little more resilience after 7 days but was not always significantly different to Svevo. Other parameters affected by increasing storage were minor decreases in springiness (ability to spring back after being deformed), larger effects for cohesiveness (ability to withstand a second deformation relative to resistance under the first deformation), and large increases in gumminess (firmness × cohesiveness) and chewiness (gumminess × springiness) (Supporting information Table S2). There were no effects on storage time trends from the influence of LV or specific volume. SvLA and even more the SvLA 5 + 10 loaves had higher LV than Svevo. Other researchers found bread containing waxy wheat flour has higher loaf volumes (Morita et al., 2002; Purna et al., 2011). Increased loaf volume can contribute to the anti-staling effect (Every, Gerrard, Gilpin, Ross, & Newberry, 1998) as we observed in SvLA and SvLA* but only at days 1 and 3 (Figures 3 & 4). Loaf specific volume can affect the rate and extent of staling, but in our study, no effects were found.

Starch retrogradation is widely recognized as a major contributor to staling but does not completely account for firming (Gray & BeMiller, 2003). We observed an increase in retrogradation enthalpy with storage time for all samples reflecting a greater difficulty in gelatinizing the starch (Figure 5). This is thought to be due to more starch retrogradation having greater resistance to gelatinization. DF addition seems to reduce enthalpy compared to 100% BF bread although not significantly except for Svevo* and SvLA 5 + 10* at 7 days being lower. In both seasons, samples Svevo and Svevo* showed no differences in enthalpy across the storage days and similarly in the SvLA and SvLA*. Comparing SvLA with Svevo in both seasons at the same staling day, there were no differences in enthalpy. This is consistent with no differences in enthalpy values between bread containing waxy wheat flour and control wheat after 7 days storage (Purna et al., 2011). The presence of 5 + 10 in the SvLA did not alter retrogradation compared to the SvLA. There were no significant differences in onset, peak, and endset gelatinization temperatures between genotypes except for an increase from day 1 to 3 (Supporting information Table S3). It seems based on other research that a decrease in starch retrogradation might explain the slight anti-staling effect of SvLA flour in the early stages of storage (1–3 days) as starch retrogradation can drive textural firming of bread (Bhattacharya et al., 2002; Gray & BeMiller, 2003), but this could not be the explanation in our samples since enthalpy values did not vary significantly. Other factors must come into play such as differences in moisture distribution between the starch and gluten networks.

4 | CONCLUSIONS

Improvements in loaf volume in weak durum with type 1 LMW-GS and HMW-GS 20, as found in Lira42, can probably be achieved adding genes coding for 2 + 12 or 5 + 10 while no improvements in bake quality were obtained in stronger durum variety Svevo with 2 + 12 but still a reduction in LV with 5 + 10 even in the absence of the *Glu-B1* subunit 7 + 8. Results depended on genotype and the complex interactions between the added *Glu-D1* subunits and background glutenin and gliadin composition during the baking process. These key *Glu-D1* subunits critical for good breadmaking in hexaploid wheat appear to have limited value in improving loaf volume and structure in durum bread especially when the proportion of durum to bread wheat flour increases above 25%. Possibly, a glutenin composition that gives a better balance of strength to extensibility is needed to allow better gas expansion to increase loaf volume. A possible alternative strategy to improve breadmaking quality is the integration of active Ax and Ay subunits, present at the *Glu-A1* locus in T. dicoccoides, as suggested by Ciaffi et al. (1995).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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