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An investigation of grain characteristics, dough quality and baking performance of perennial wheats from contrasting parentage

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ABSTRACT

Perennial grains are being developed to improve the environmental sustainability of grain production systems. However, to maximise their commercial viability, a clearer understanding of their food processing properties is required. In this study, the functional properties of selected perennial wheat breeding lines grown at sites in central New South Wales, Australia, were compared to each other and an annual bread wheat cultivar. Lines were assessed for grain yield parameters, rheological properties (wholemeal and refined flour), starch properties, milling yield and refined flour baking quality. Perennial wheats were found to differ from expected behaviours attributed to annual wheat, offering novel combinations of grain characteristics. Despite softer grain and rheological tests indicating only moderate gluten strength, several lines exhibited better baking performance than the conventional bread wheat control. Furthermore, flour water absorption was found to decrease with increasing grain hardness, the opposite of that normally observed for annual wheat. The results demonstrated that with appropriate breeding and selection, perennial wheat offers good potential for baking.

1. Introduction

Perennial wheats (PW) are derived from crosses between either common wheat (*Triticum aestivum*) or durum wheat (*T. durum*) and wheatgrass (*Thinopyrum* spp.), with original breeding efforts occurring in Russia in the 1920's (Wagoner, 1990). Perennial crops are of interest due to their sustainability and environmental attributes – primarily the improved resource-use efficiency attributable to longer growing seasons (Zhang et al., 2011; Crews et al., 2016) and multifunctionality (Ryan et al., 2018) compared to annual crops. Perennial crops also mitigate soil degradation through reduced soil disturbance, promoting year-round vegetative cover and increased utilisation of soil water, with their extensive root biomass development accumulating carbon in soils (Shi et al., 2011). A diversity of PW breeding lines have been evaluated across a range of locations in recent years (Hayes et al., 2012, 2018; Larkin et al., 2014). Trials in Australia have largely focused on PW potential as a dual-purpose crop, providing grazing of forage and opportunistic grain production for lower input cost (Newell and Hayes 2017; Hayes et al., 2017).

Grain of PW has also been shown to possess several human health claimable compounds compared to annual cereal grains including greater concentrations of bioactive compounds (yellow pigments, polyphenols and resorcinolic lipids), dietary fibre and resistant starch (Pogna et al., 2014). This provides an opportunity for development of perennial grain food products with health and nutritional benefits for consumers. However, little is known about its baking performance and other functional traits. Lachuga et al. (2023), reported on a single perennial wheat variety "Pamyati Lyubimovoy", referred to as

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Trititrigia, grown in the southern Rostov region of Russia and found it to have poorer baking quality compared to a winter wheat, while an initial study of F_5 PW breeding lines derived from a *Th. ponticum*/Chinese Spring//Madsen[1] population indicated the perennial lines had inferior milling yield and baking and loaf characteristics compared to bread wheat (Murphy et al., 2009). To the best of the authors knowledge, there is no published literature on comparative milling, rheological and baking qualities for a broader range of PW pedigrees. The present study investigates the year one grain characteristics and functional properties of a diverse range of PW lines grown in central New South Wales (NSW), Australia, during 2018 and 2019.

2. Materials and methods

2.1. Experimental sites & germplasm

Four field experiments were established at Cowra (site A, C18) and Mandurama (M18) in 2018; and in 2019 at Cowra (site B, C19) and Orange (O19) in NSW, Australia. Each experiment comprised small plots (2 × 7.5 m) in randomised block designs with three replicates. Seven PW genotypes and the winter wheat cultivar, Wedgetail, were trialled at each site (Table 1). Wedgetail wheat was included as an annual wheat control entry. Wedgetail is a variety typically grown in southern Australia and classified as an Australian Prime Hard (APH) variety. APH varieties are hard grained, quality milling wheats with strong and balanced dough properties (Grains Australia 2023). The PW lines were partial amphiploids between wheat (*Triticum* spp.) and wheat grass (*Thinopyrum* spp.) and represented a diversity of parentage and were selected based on post-harvest regrowth performance in previous field assessments (Hayes et al., 2012, 2018; Larkin et al., 2014). The 2019 harvest year experienced drier conditions than 2018, receiving less rainfall during the flowering period but minimum and maximum temperatures recorded in all experimental sites were relatively similar to the long-term average (Fig. 1).

Grain was harvested from each plot in each experiment at the end of year one. Due to lower yields of some genotypes, and the severe drought year of 2019, sufficient quantities of grain were not available for all tests. As a consequence, three sets of samples were used at various stages in this study, dictated by grain availability or suitability: Set A (all entries from both 2018 and 2019 sites); Set B (all entries at 2018 sites only, composite samples of grain from each genotype at each site); and Set C (2018 sites only, composite samples of four perennial lines, plus Wedgetail). It is noted that there is no replication in the data presented from Set C.

Table 1
Identification (ID) and pedigree details of genotypes.

ID	Pedigree
11955	*Triticum aestivum/Thinopyrum ponticum*
20238	*T.durum (S)/Th.elongatum*
CPI-235a (235a)	*T.aestivum (CS)/Th.ponticum/T.aestivum (M)*
CPI-147251b (251b)	*T.aestivum (CS)/Th.ponticum/T.aestivum (M)*
OK7211542 (OK72)	*T.aestivum/Th.ponticum*
Otrastajuscaja 38 (Ot38)	*T.aestivum/Th.intermedium*
Summer 1	*T. aestivum/Th.intermedium*
Wedgetail	*T.aestivum*

Chinese Spring (CS) and Madsen (M) are cvv. of *T.aestivum*; Stewart (S) is a cv. of *T. durum*.

[1] Murphy et al. (2009) initially described this pedigree as *Th. elongatum*/Chinese Spring//Madsen, but Curwen-McAdams and Jones (2017) later clarified the perennial parent as being *Th. ponticum*.

2.2. Set A analyses: agronomic performance, grain attributes and wholemeal mixograph

Anthesis date was recorded for each genotype at each site and expressed as days after sowing (DAS). Harvest and grain yield sampling were targeted to the maturity of genotypes. Once each genotype had ripened, a representative sample of biomass was removed from each plot by cutting two adjacent rows at ground level, along a 50 cm ruler (total area = 0.36 m^2) at either end of the plot. This sample was weighed, and grain removed to determine the proportion of grain to total biomass produced (harvest index, HI). All remaining grain heads were removed from each plot by hand. This material was then threshed and cleaned with a stationary thresher (Kimseed, Perth, Australia) from which final grain yield (kg/ha) was determined for each plot.

Grain length and width (mm) were measured with digital callipers on three randomly selected grains from each plot. Length was measured from the germ to the brush end of each kernel, and width measured from dorsal to ventral (crease) side (DV) and also from left to right (LR) of each kernel. Grain weight (g) was determined in triplicate by counting 100 grains (Contador seed counter, Pfeuffer GmbH, Kitzingen, Germany), weighing to 3 decimal places and multiplying by 10; reported as thousand kernel weight (Kwt). Grain colour was measured in triplicate using a CR-400 chromameter (Minolta, Tokyo, Japan) and reported in CIE colour space L* a* b* where L* represents brightness, a* redness, and b* yellowness. Total grain protein (GP) was determined by placing 5 g of grain from each plot into a ball mill (MM200, Retsch, Haan, Germany) for 1 min at 25 hz. Samples were then sifted (1 mm sieve) and stored at room temperature until analysed. Nitrogen content was determined in triplicate using a CHN analyser (2400 series II, PerkinElmer, Waltham, MA, USA) and converted to protein content (not adjusted for moisture content) by applying a multiplication factor of 5.7 (Mariotti et al., 2008).

For mixograph analysis, approximately 10 g from each field plot was combined to provide a single sample per site of each genotype. Grain was ground in a hammer mill (3100 laboratory mill, Perten Instruments, Macquarie Park, NSW, Australia) fitted with a 0.8 mm sieve, and moisture content determined in duplicate by heating approximately 1.5 g meal to 130 °C in a thermo-gravimetric analyser (TGA701, Leco Corporation, St Joseph, MI, USA), and holding until constant weight achieved. Adjusting sample weight to 11 % moisture basis (mb), samples were then analysed in duplicate using a 10 g mixograph (National Manufacturing, Lincoln, NE, USA) and Mixsmart for Windows software V1.0.404 according to Approved Method 54-40.02 (AACC, 2010). The standard equation to determine water addition was modified through trial and error to account for the use of wholemeal (rather than refined flour) and to achieve midline peaks in the desired mid-chart range (50–65%) for mixograph analysis. Thus, PW received (% water) 1.5*protein content of sample + 41, while Wedgetail wheat received 1.5*protein content + 46. Reported parameters were peak time, peak height, peak width, peak integral (peak torque x mins), width and height at 5.5 min, breakdown (defined as peak height minus height at 5.5 min), and descending slope (defined as breakdown/5.5 minus peak time).

2.3. Set B analyses: grain hardness, moisture and ash content

Grain hardness was determined (in duplicate) as particle size index (PSI) according to Symes (1961) utilising a disc grinder at the finest setting (Buhler-Miag, Uzwil, Switzerland) and an EFL2000/1 sieve shaker (Endecotts Ltd, Sth Wimbledon, UK) fitted with 75 μm sieves. Higher numbers indicate softer grains. Ash and moisture content (duplicate) was determined using approximately 1.5 g meal in a thermo-gravimetric analyser (TGA701, Leco Corp., USA). Samples were dried to constant weight at 130 °C to determine moisture content, then ramped to 600 °C and again held until constant weight to determine ash content (reported on 11% mb).

2.4. Set C analyses: grain, flour and dough characteristics

Test weight was determined in duplicate using a mini chondrometer (Wagga Wagga Agricultural Institute, Australia) calibrated to a Franklin chondrometer (Franklin Instruments, Sydney, NSW, Australia) and reported as kg per hectolitre. Kernel weight (duplicate) was determined as described for Set A except 250 grains were counted and the weight multiplied by four. Whole grain and wholemeal colour were measured in duplicate using a CR-410 chromameter (Minolta, Japan) fitted with a 50 mm head and granular materials attachment (CR-A50), reported in CIE colour space L* a* b* as described above.

Duplicate 150 g grain samples were conditioned to approximately 12.5% moisture and milled in a Quadrumat junior mill (Brabender, Duisburg, Germany) in randomised order. Flour extraction was determined as the flour weight as a percentage of total products (bran plus flour). Flour samples from mill duplicates were then combined for all remaining tests.

Flour moisture and ash were determined as described for Set B, with ash reported on 14% mb. Flour nitrogen content was determined in triplicate using a Trumac (Leco Corp., USA) standardised with EDTA, and protein (N% x 5.7) was reported on 14 % mb. Flour colour was determined as described for wholemeal. Flour total starch content was determined in duplicate using the rapid total starch method K-TST (Megazyme, Bray, Ireland) and reported on a dry matter basis. Starch pasting parameters of initial gelatinisation temperature, peak viscosity, breakdown, final viscosity and setback were obtained using standard 1 profile on S4 rapid visco-amylograph (RVA) (Perten Instruments, Australia). Rheological tests were performed in duplicate using a 4 g DoughLAB (Perten Instruments, Australia) mixing at 150 rpm to a target peak of 630 farinograph units. Water absorption, time to peak (dough development time = DDT) and stability were reported according to AACC Approved Method 54-21.02 (AACC, 2010), as well as width at peak, energy (work input) to peak and softening (decrease in midline peak height) at 3 min after peak. Flour mixographs were performed in duplicate as described for Set A, using flour on 14 % mb and % water addition equation of 1.5*protein content +45.3 for all samples.

Straight dough baking formulation comprised 50 g flour (100 %), 1.5 % salt, 1 % sugar, 0.1 % ammonium chloride, 1.1 % Lesaffre red label instant yeast and 0.5 % Saunders malted barley extract. Bakery water addition was taken from DoughLAB water absorption without adjustment. Baking was performed in duplicate, yielding 4 loaves per genotype. Doughs were mixed to development at 150 rpm (DoughLAB 2500, Perten Instruments, Australia), then scaled to 2 × 40 g dough pieces and fermented for a total of 75 min at 30 °C, with a first knock and mould at 60 min (80% total ferment time). Each dough was then lightly knocked down and moulded again in a bun moulder (Domex, Dewsbury, UK) before placing in open square bake tins and proofing for 45 min at 34 °C and 85% relative humidity. Loaves were baked at 214 °C in a bakery oven (Rotel 2, Moffatt, Melbourne, VIC, Australia) for 15 min. After removal from the oven, loaves were turned out of tins and cooled for 45 min before determining weight and (duplicate) volume (seed displacement). Specific volume was reported as weight divided by volume. Loaves were stored overnight before subjective assessment of external appearance and internal crumb structure, instrumental crumb colour (quadruplicate per loaf) and crust colour (duplicate) using a 210 chromameter fitted with an 8 mm head (Minolta, Japan), and instrumental crumb texture (single 20 mm thick slice per loaf) using a texture analyser (TATX2, Stable Microsystems, Godalming, UK) fitted with a 25 mm cylindrical head according to TA.XTPlus application study BRD2/P36R (Stable Micro Systems, 2006).

2.5. Data analysis

Data were analysed using linear models and ANOVA using the R language (R Core Team, 2020). For harvest index, the data were natural log-transformed to reduce heteroskedacity. Grain yield, 1000 kernel weight, harvest index, anthesis date and protein concentration were analysed using a linear model with the agronomic variable as the explanatory variable and genotype and site/year and the interaction between genotype and site/year as the fixed variables.

For Sets A and B data where composite samples of each genotype were used for laboratory testing (see Tables 3 and 4), ANOVA with genotype as the fixed variable and site/year as the random variable was conducted in Genstat v. 20 (VSN International), with least significant differences calculated at $P = 0.05$. The baking and flour parameters (Set C) were not analysed statistically but were described qualitatively with presented data for each genotype representing the average of duplicate or triplicate samples (see Tables 5–8).

3. Results

3.1. Agronomic performance and grain attributes

Averaged across all site/years, the year 1 grain yield of Wedgetail wheat was 4-fold greater than the PW breeding lines (Table 2). Among PW, Summer 1 had the highest grain yield, although this was not significantly higher than 11955 at $P = 0.05$. A similar trend was observed in grain weight, with 11955 and Summer 1 both having a higher kernel weight than the remaining perennial lines, but both lower than Wedgetail wheat. The perennial lines 251b, 235a and Ot38

Table 2
Significant main effects of genotype and site/year on grain yield, harvest index (HI), thousand kernel weight (Kwt) and grain protein (GP).

Treatment	Grain yield (kg/ha)	HI (%)	Kwt (g)	GP[a] (%)	Anthesis (DAS)[b]
Genotype main effect ($P < 0.001$)					
11955	806	17.8	28.5	19.0	182.1
20238	712	16.5	25.4	19.8	174.8
235a	654	11.7	21.7	19.7	190.6
251b	603	12.2	22.4	20.7	191.5
OK72	753	12.6	25.8	20.1	180.6
Ot38	651	10.9	20.6	20.6	201.0
Summer 1	966	26.5	27.8	18.6	156.1
Wedgetail	3219	28.9	33.2	17.4	153.5
$l.s.d._{0.05}$	*168.3*	*1.94*	*1.16*	*0.56*	0.79
Site/year main effect ($P < 0.001$)					
Cowra 2018	1515	26.3	27.9	18.7	162.4
Cowra 2019	567	0.9	23.0	20.8	187.6
Mandurama 2018	1455	30.4	29.7	18.4	187.1
Orange 2019	645	11.0	22.1	20.0	178.0
$l.s.d._{0.05}$	*119.0*	*1.37*	*0.82*	*0.40*	0.56

[a] Not corrected for moisture content.
[b] Days after sowing.

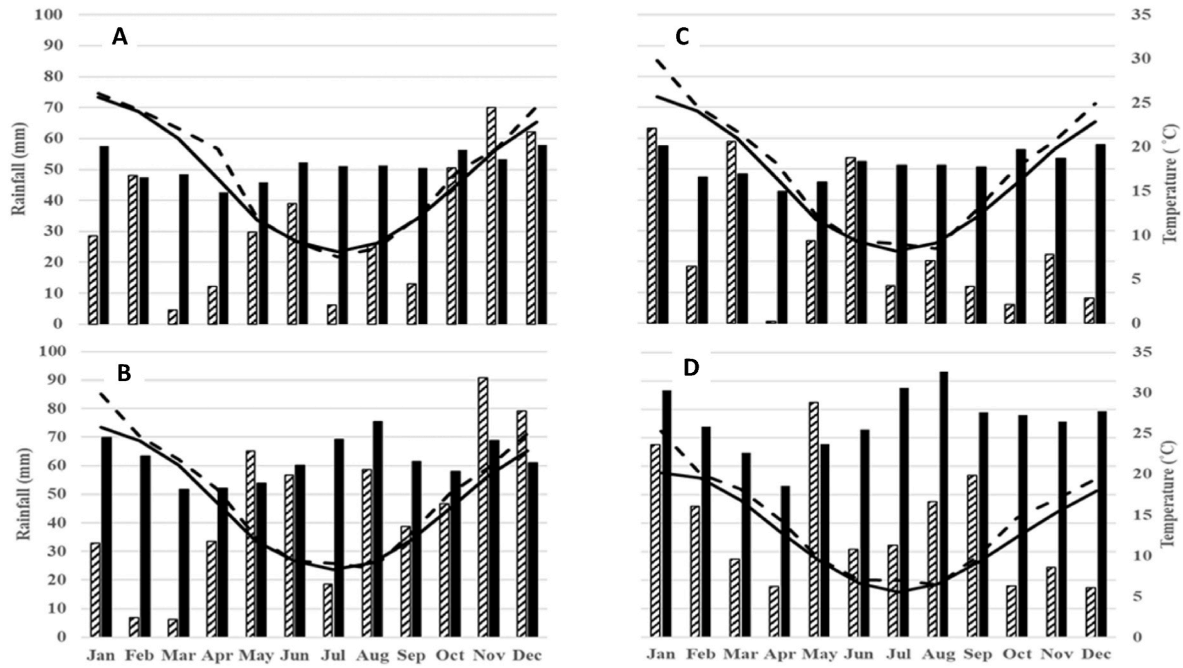

Fig. 1. Mean monthly temperature (▬) along with monthly long-term average (▬) as well as total monthly rainfall (▨) and the monthly long-term average (■) recorded at each site or nearby weather station. A = Cowra 2018; B = Mandurama 2018; C = Cowra 2019; D = Orange 2019.

generally had the lowest grain yield and harvest index of all lines tested. The harvest index of Summer 1 (26.5%) approached that of Wedgetail (28.9%), with both substantially higher than all other PW lines tested (average 13.6%). All perennial lines were longer in their maturity, which was reflected in the number of days from sowing to anthesis (DAS) (Table 2). There was a strong negative correlation between the average time to anthesis and average grain yield of all genotypes at each site ($R^2 = 0.52$, $P < 0.001$, $n = 32$). Earlier flowering lines tended to have higher grain yields (e.g. Summer 1, 156 DAS) compared to later maturing lines (e.g. Ot38, 201 DAS). Yields, harvest index and grain size were all lower in the 2019 experiments compared to 2018, reflecting much drier seasonal conditions (Fig. 1). This, in turn, influenced protein content with higher GP values in 2019 and a significant negative correlation between GP and Kwt ($R^2 = 0.56$, $P < 0.001$).

PW lines were, on average, 11% longer in grain length compared to Wedgetail wheat (Table 3). In contrast, Wedgetail was wider in both DV and LR width measurements compared to PW lines. Some variation in grain colour was observed between the various perennial genotypes. The perennial line 20238 was characterised as significantly less yellow (b*) and less bright (L*) than all other genotypes, while 11955 and OK72 had a higher degree of redness (a*) compared to most other entries. Ash content was, on average, 42 % higher in the PW entries compared to Wedgetail.

3.2. Grain hardness and flour attributes

Grain hardness (PSI, determined for 2018 sites only) varied greatly among genotypes, with 20238 recording equivalent PSI to Wedgetail and characterised as medium hard. The highest PSI (softest grain) was recorded for 11955, characterised as extra soft (Table 4). Grain moisture was similar between all lines, ranging from 9.5 to 9.8 % (Table 4). Grain protein (GP, 11 % mb) was lower in Wedgetail (16.1 %) than all PW lines, although not significantly different to Summer 1, whilst the highest protein concentration was observed in 251b and OK72 (Table 4). Mixograph water addition for individual sites ranged from 66.1 % (Summer 1 at C18) to 72.8 % (Wedgetail at O19) and was on average (across sites) 0.66 % lower per protein unit for PW compared to Wedgetail to achieve the equivalent target peak height of 50–65 %. Lowest peak height was 52.9 % for 20238, and highest (65.5 %) for OK72. Time to peak was significantly lower for PW in comparison to Wedgetail ($P < 0.001$), except 235a which had an extended peak time, on average 47% higher than all other PW lines ($P < 0.001$). Peak width

Table 3
Grain length, dorsal-ventral width (DV wd), left-right width (LR wd), colour (brightness L*, redness a*, yellowness b*) and ash of perennial lines compared to Wedgetail wheat, determined from grain produced at the four experimental sites in 2018 and 2019.

Genotype	Length mm	DV wd mm	LR wd mm	Grain colour			Ash*
				L*	a*	b*	%
11955	7.01	2.04	2.37	48.3	5.1	14.0	1.89
20238	7.71	1.93	2.27	45.6	4.4	10.5	1.75
235a	5.94	1.99	2.32	49.3	4.5	13.6	1.96
251b	6.62	1.79	2.28	48.0	4.9	14.2	2.02
OK72	6.85	2.06	2.36	48.9	5.1	14.2	2.07
Ot38	6.48	1.92	2.09	49.2	4.8	13.3	1.91
Summer 1	6.60	2.14	2.51	47.6	4.9	13.5	1.53
Wedgetail	6.06	2.40	2.73	49.2	4.4	14.0	1.32
l.s.d$_{0.05}$	*0.34*	*0.15*	*0.15*	*1.2*	*0.4*	*0.8*	*0.16*
	<0.001	*<0.001*	*<0.001*	*<0.001*	*= 0.001*	*<0.001*	*<0.001*

DV wd = dorsal ventral width; LR wd = left-right width; Ash = ash at 11% moisture basis, *determined on 2018 entries only.

Table 4
Grain hardness (PSI), wholemeal moisture, protein and mixograph parameters of perennial lines compared to Wedgetail wheat, determined from grain produced at the four experimental sites in 2018 and 2019.

Genotype	PSI	PSI	GM	GP	Mw	PkT	PkH	PkW	PkI	5.5H	5.5W	BDn	DSl
		descriptor	%	%	%	min	%	%	Tq*min	%	%	%	%/min
11955	41	Extra soft	9.8	18.4	68.5	1.5	62.4	30.5	73	48.0	7.5	14.5	3.5
20238	17	Med hard	9.5	18.4	68.6	1.4	52.9	19.9	59	35.6	14.3	17.3	4.2
235a	24	Med soft	9.8	18.3	68.4	2.9	63.4	31.0	143	54.5	17.3	8.8	3.3
251b	30	Soft	9.7	19.4	70.1	1.4	57.1	24.9	65	42.6	4.8	14.3	3.5
OK72*	34	Very soft	9.6	19.4	70.0	1.6	65.5	29.8	84	50.4	3.9	14.6	3.9
OT38*	23	Med soft	9.6	19.3	69.9	1.3	57.4	24.2	57	39.4	1.7	17.7	4.2
Summer 1	27	Soft	9.6	17.3	67.0	1.9	58.5	27.8	88	43.4	12.9	15.1	4.1
Wedgetail	17	Med hard	9.8	16.1	70.2	2.6	60.3	26.9	125	51.5	12.5	8.9	3.0
$l.s.d_{0.05}$	*4.5*	-	*ns*	*1.2*	*1.72*	*0.38*	*4.39*	*3.07*	*17.2*	*7.24*	*8.17*	*6.26*	*ns*

PSI = particle size index (determined on 2018 sites only); GM = Grain moisture; GP = grain protein 11% mb; Mw = mixograph water addition; PkT = mixograph time to peak; PkH = peak height; PkW = peak width; PkI = peak integral; 5.5H = height at 5.5 min; 5.5W = width at 5.5 min; BDn = breakdown; DSl = descending slope; ns, differences not significant at $P = 0.05$.*OK72 lacks mixograph data from C19 site; Ot38 lacks data from C19 and O19 sites.

Table 5
Flour extraction, protein, ash, and total starch; RVA parameters; and DoughLAB parameters of three PW genotypes compared to Wedgetail wheat using grain from two sites sown in 2018.

Genotype	FE	FP	Ash	TS	Pv	Fv	Sb	WA	DDT	Stab	Soft3	PkE
	%	%	%	%	cP	cP	cP	%	min	min	FU	Wh/kg
Cowra18												
235a	48.6	15.1	0.58	73.6	1980	2448	1140	59.5	2.23	1.9	108	24.1
251b	53.0	17.0	0.48	72.6	2652	2748	996	61.3	0.91	0.7	223	19.7
11955	55.1	16.3	0.45	75.4	1632	1824	876	62.9	1.25	1.1	170	21.8
Wedgetail	66.1	13.5	0.45	78.1	2340	3132	1464	59.7	2.68	2.4	118	24.0
Mandurama18												
235a	50.1	16.3	0.63	72.8	1500	1908	948	59.2	2.11	2.1	103	24.1
251b	52.5	17.9	0.58	73.0	2316	2604	1068	61.5	0.83	0.5	285	18.8
OK72	54.6	17.8	0.50	68.5	2052	2376	1080	60.5	1.25	1.2	150	23.0
Wedgetail	64.6	13.9	0.45	77.6	2796	3180	1476	59.0	3.05	3.1	85	24.4

FE = flour extraction; FP = flour protein 14% mb; TS = total starch; Pv = RVA peak viscosity, in centipoise (cP); Fv = final viscosity; Sb = setback; WA = DoughLAB water absorption; DDT = dough development time; Stab = stability; Soft3 = softening at 3 min past peak, in Farinograph Units (FU); PkE = total energy to peak (watt hours per kg). (Data not replicated).

was also highest in 235a, which along with 11955, was greater than for Wedgetail ($P < 0.001$). Greatest peak torque integral value was recorded for 235a, which was 50% higher than all other PW lines, and similar to Wedgetail ($P < 0.001$). Lowest breakdown and descending slope were observed in Wedgetail and 235a, and greatest in 20238 and Ot38 (Table 4).

3.3. Milling, rheology and baking performance

Milling was performed on Set C, comprising lines chosen on the basis of wholemeal mixograph results to cover a range of dough strength types. Lines 235a, 251b, and Wedgetail were included from both sites in 2018, however, 11955 and OK72 were from the 2018 sites at Cowra and Mandurama, respectively. Flour extraction was, on average, ~20 % higher for Wedgetail than the PW genotypes (Table 5). DoughLAB rheology parameters indicative of dough strength (development time, stability, and work to peak) were also higher for Wedgetail compared to PW genotypes, of which 235a exhibited the highest values for these parameters and 251b the lowest (Table 5). Within PW genotypes, DoughLAB water absorption was associated with higher (softer) PSI. Starch pasting curves of PW, while typical of wheat flour, were generally of lower peak viscosity, final viscosity, and setback than Wedgetail, with 251b displaying greatest peak and final viscosity amongst PW lines (Table 5). In flour mixographs, 235a was again identified as the PW genotype consistently exhibiting stronger dough parameters, while 251b recorded the lowest peak time, peak integral and width at 5.5 min

Table 6
Flour mixograph parameters of three PW genotypes compared to Wedgetail wheat using grain from the two sites sown in 2018.

Genotype	Mw	PkT	PkH	PkW	PkI	5.5H	5.5W	BDn	DSl
	%	min	%	%	Tq*min	%	%	%	%/min
Cowra18									
235a	67.9	4.0	53.4	24.8	171	51.5	15.8	2.0	1.4
251b	70.1	1.4	54.3	22.7	63	42.7	3.7	11.6	2.8
11955	69.7	1.8	58.8	28.6	87	47.3	4.6	11.6	3.2
Wedgetail	65.5	4.6	56.2	24.4	206	54.3	20.6	1.9	2.0
Mandurama18									
235a	69.7	3.9	57.8	25.9	169	53.3	9.3	4.5	2.7
251b	72.1	1.3	49.9	21.5	55	38.3	2.7	11.6	2.8
OK72	72.1	2.2	56.3	21.1	98	45.7	5.2	10.5	3.1
Wedgetail	66.2	5.0	59.7	27.1	227	58.7	26.8	1.0	1.6

Mw = mixograph water addition; PkT = mixograph time to peak; PkH = peak height; PkW = peak width; PkI = peak integral; 5.5H = height at 5.5 min; 5.5W = width at 5.5 min; BDn = breakdown; DSl = descending slope. (Data not replicated).

Table 7
Colour of whole grain, wholemeal and flour, of three PW genotypes compared to Wedgetail wheat using grain from the two sites sown in 2018 (brightness L*, redness a*, yellowness b*). (Data not replicated).

Genotype	Whole grain colour			Wholemeal colour			Flour colour		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
Cowra18									
235a	56.9	5.7	15.3	82.8	0.9	11.8	91.5	−2.1	10.3
251b	56.8	7.1	17.6	84.0	0.5	11.8	92.0	−2.3	10.7
11955	58.8	6.4	16.5	85.0	0.6	10.6	92.5	−2.1	9.1
Wedgetail	61.2	6.0	18.9	83.2	1.1	12.6	90.3	−1.4	10.0
Mandurama18									
235a	57.8	5.9	15.9	83.4	0.6	11.5	91.3	−1.9	9.6
251b	55.9	6.8	16.4	83.6	0.7	11.4	91.2	−2.0	10.0
OK72	55.9	6.5	16.3	83.0	0.9	10.5	91.7	−1.9	8.9
Wedgetail	57.3	6.2	16.4	82.9	1.3	12.4	90.1	−1.2	9.6

Table 8
Bake test parameters of three PW lines compared to Wedgetail wheat using grain from the two sites sown in 2018.

Genotype	BWA	Mix	LV	SpV	ExtA	IntS	Firm	Crumb colour			Crust colour		
	%	min	cc	cc/g	of 5	of 5	gF	L*	a*	b*	L*	a*	b*
Cowra18													
235a	63.6	3.15	125	3.75	4.3	3.0	511	69.1	−5.6	22.9	58.3	8.2	35.7
251b	63.7	1.75	128	3.94	3.0	3.0	383	69.2	−6.3	23.0	58.4	7.6	36.7
11955	66.9	2.25	129	4.00	3.5	4.0	381	71.1	−6.3	20.5	60.2	7.5	38.1
Wedgetail	63.5	3.65	130	3.92	4.0	4.5	523	72.0	−5.6	17.0	63.2	6.2	36.8
Mandurama18													
235a	63.4	2.95	129	3.88	4.5	2.5	480	68.7	−5.2	21.8	55.2	9.3	34.1
251b	64.1	1.50	114	3.49	2.0	1.0	487	66.0	−5.2	21.7	54.7	9.4	35.1
OK72	64.6	2.35	132	4.06	5.0	5.0	441	70.8	−5.5	19.6	56.4	9.1	36.6
Wedgetail	62.8	4.30	127	3.86	4.0	5.0	337	71.4	−5.3	16.8	62.6	6.0	36.0

BWA = bakery water absorption; Mix = bakery mix time; LV = loaf volume; SpV = specific volume; ExtA = external appearance score; IntS = internal structure score; Firm = crumb firmness (gF = grams force); L*,a*,b* = crumb and crust colour. (Data not replicated).

indicating weaker dough strength (Table 6; Fig. 2). Total starch contents, while lower for PW than Wedgetail, were all above 72 % apart from OK72 (68.5 %) and showed a negative trend against flour protein content across all entries (Table 5).

At each site, Wedgetail flour colour was less bright (L*) and more red (a*) than the PW entries. Notably 251b was more yellow (b*) than all other lines. Grain colour was not a good indicator of wholemeal or flour colour, particularly redness, however flour colour was better predicted by wholemeal colour, especially brightness and yellowness (Table 7).

Averaged duplicate results (4 loaves) of baking tests are shown in Table 8. Bakery mix time ranged from 1.5 min for 251b to 4.3 min for Wedgetail, and loaf volume from 114 cc (251b at M18) to 132 cc (OK72 at M18). Wedgetail, 11955 and OK72 exhibited very good internal crumb structure, while external loaf appearance of 251b at M18 was judged to be unsatisfactory (Fig. 3). From grain produced at Cowra in 2018, PW lines 11955 and 251b produced softer (average 382 gF) crumb than Wedgetail (523 gF), while at Mandurama in the same year Wedgetail (337 gF) was softer than all perennial lines (average 469 gF). Crumb brightness (L*) was consistently greatest for Wedgetail, and 251b from Cowra was noted as being much brighter compared to that from Mandurama. Crumb yellowness (b*) and redness (a*) were lower in Wedgetail than all PW, while crust L* was greater. Loaf internal and external character is shown in Fig. 3.

4. Discussion

4.1. Agronomic performance and grain attributes

Grain yields varied between the four sites and were largely affected by environmental conditions during the growing season. The experimental year of 2018 was characterised by lower-than-average rainfall which had a negative impact on grain yields at both Cowra and Mandurama, especially for later-maturing lines that struggled to fill grain under dry conditions. A worsening of drought conditions in 2019 impacted perennial wheat yields even more than in 2018. Grain weight, a direct function of dry matter accumulation during grain filling (Xie et al., 2015), was on average 27 % lower than in 2018 reflecting the truncated grain filling period. Phenology plays an important part in determining wheat grain yield as quicker maturing genotypes can have up to a 16 % yield advantage under conditions in which growing season rainfall is constrained (Qaseem et al., 2019; Cann et al., 2023). In the current study, 52% of the variation in yield was explained by time to anthesis. In general, grain yields were higher for the annual cereal (Wedgetail) which was the earliest to maturity at each site. The better yielding perennial wheat lines were Summer 1 and 11955 (30 % and 25 % of the yield of Wedgetail across sites respectively), which had consistently higher grain yields than the other perennial genotypes and earlier flowering times. Bell (2013) suggested that PW lines must achieve 40% of the grain yield of annual wheat to reach equivalent profitability in a mixed farming system, accounting for the additional biomass from PW production that is available for grazing. The higher yielding PW lines in this study approached this 40% yield target, and a subset of the same PW lines were reported to reach this yield target over a two-year period in a defoliation study conducted by Newell and Hayes (2017).

4.2. Milling extraction and hardness

As expected, Wedgetail had much greater flour extraction than all other entries, being 11–17.5 % higher than the best and poorest PW, respectively. This differential is consistent with Murphy et al. (2009), where extraction reported for PWs derived from their *Th. ponticum*/Chinese Spring//Madsen population were between 12.3 and 16.2 % lower than annual wheat. Test weight and kernel weight were also substantially greater for Wedgetail in the present study. Test weight is generally regarded as an indicator of milling quality and has also been

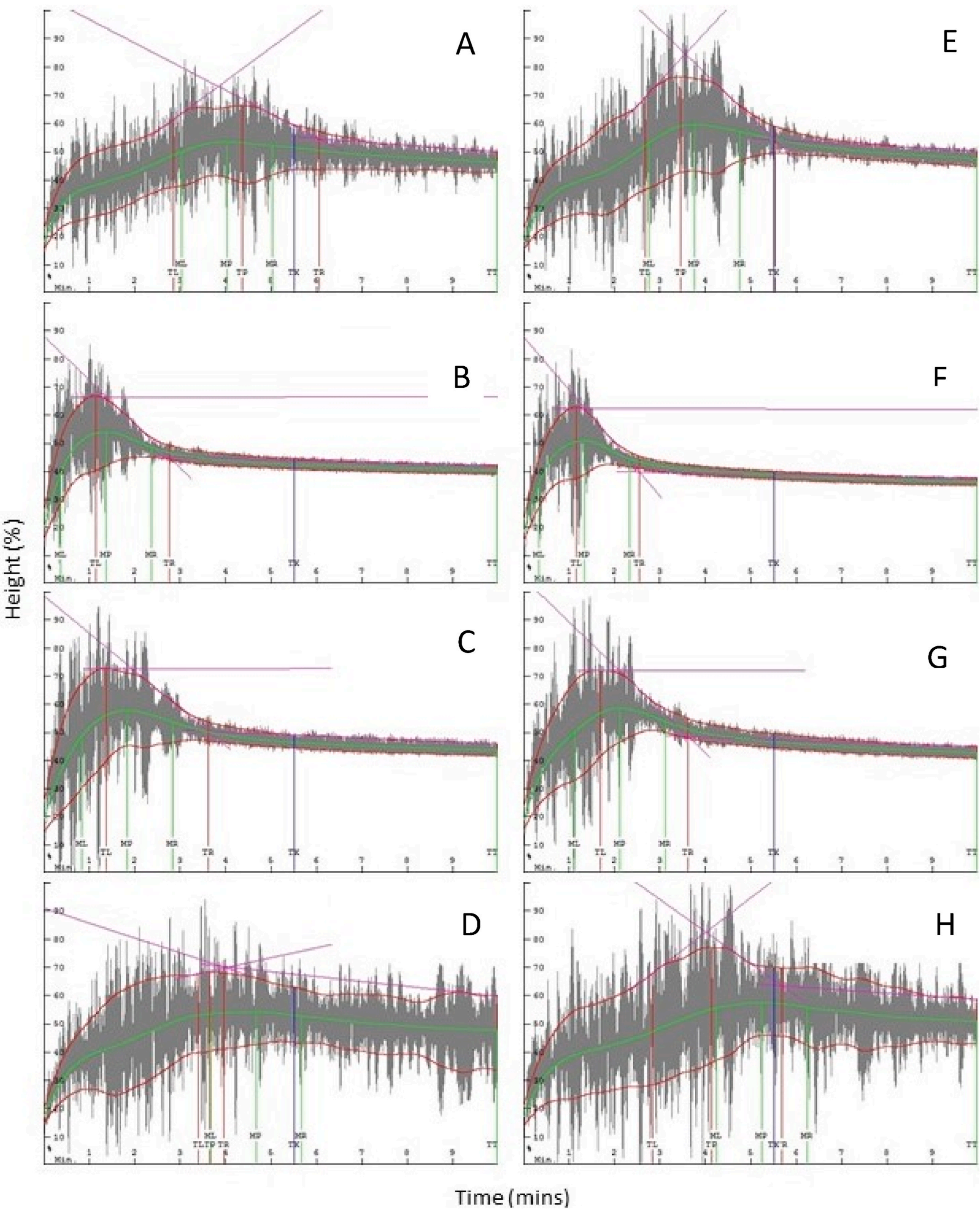

Fig. 2. Flour mixographs: A, B, C, D = grain from Cowra18, genotypes 235a, 251b, 11955, Wedgetail. E, F, G, H = grain from Mandurama18, genotypes 235a, 251b, OK72, Wedgetail.

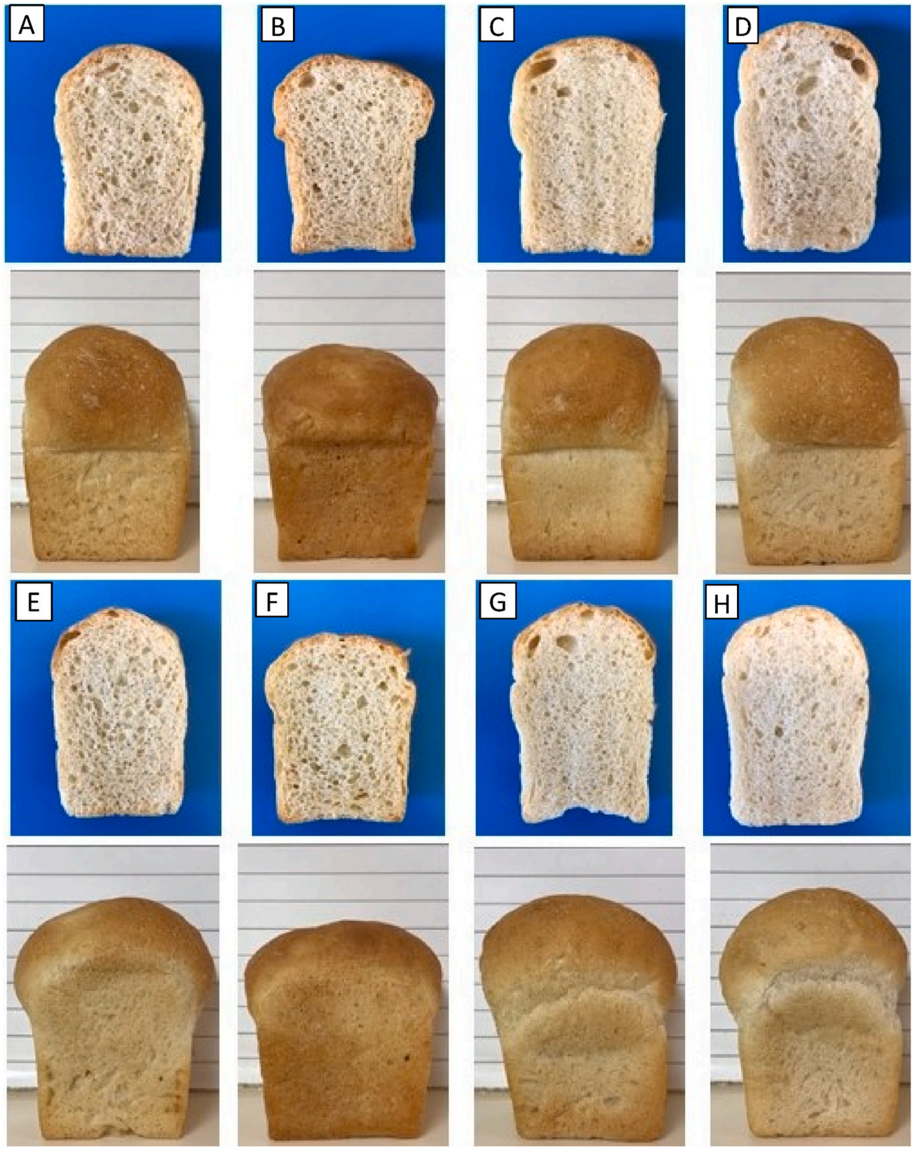

Fig. 3. Internal and external appearance of loaves: A, B, C, D = grain from Cowra (2018), genotypes 235a, 251b, 11955, Wedgetail. E, F, G, H = grain from Mandurama (2018), genotypes 235a, 251b, OK72, Wedgetail.

found to have a positive relationship with kernel weight, however the evidence for either claim is not conclusive (Dziki and Laskowski, 2005; Wang and Fu, 2020). Grain of PW lines were generally longer and narrower than Wedgetail. Lower flour extraction in PW compared to common wheat may be attributed to selective breeding in the latter to achieve larger spherical features i.e., shorter length and greater width (Gegas et al., 2010), effectively increasing the endosperm to bran ratio. In wheat, higher extraction is associated with harder grain character, governed by the presence of puroindoline alleles (Martin et al., 2007) and is attributed to ease of separation of bran from endosperm and greater sifting efficiency of the released flour (Williams, 2011; Delcour and Hoseney, 2010). While several studies involving manipulation of puroindoline alleles in hard wheats (e.g. Martin et al., 2001; Wanjugi et al., 2007) and in very hard (durum) wheats (e.g. Gazza et al., 2011; Murray et al., 2016) have found a correlation between decreased kernel hardness and increased flour yield, there is not consistent evidence for the same relationship in soft wheats. In studies of US soft wheats, Baenziger et al. (1985) found poor correlation between PSI and flour yield, and Morris et al. (2004), found club wheats to have the hardest texture and highest flour yield, whilst the lowest yielding cultivar of all entries was also the softest. Choy et al. (2015) investigated relationships between kernel hardness and milling yield in Australian wheats. Although the number of soft lines included in a VIDA historical dataset were small, there was no link between kernel hardness and flour yield for those lines either, across or within growing environments. In our study, a strong trend was observed between hardness (PSI) and extraction. With a PSI value of 41 (extra soft), 11955 was the best milling PW, while 235a, the “hardest” PW milled (PSI of 24, medium soft) had the lowest extraction. It is worth noting that 235a shares a similar origin to the material tested by Murphy et al. (2009), who also observed low flour extraction in their PW material.

Kernel hardness in wheat is controlled by puroindoline genes located on the D chromosome, commonly referred to as PIN genes, which govern the degree of adhesion between starch granules and the protein matrix, thus influencing milling behaviour (Chen et al., 2007; Pasha et al., 2010). Soft PW lines such as 251b, OK72 and 11955 possess wild type alleles PINa-D1a and PINb-D1a inherited from common wheat, which code for softness (Pogna et al., 2014; Gazza et al., 2016). However, 235a was found to possess novel previously undescribed PIN-A and PIN-B alleles (presumably inherited from the perennial parent) and this may have influenced the milling behaviour observed in this study. Murphy et al. (2009) found PW on average produced softer grains than the annual control wheat, and with the exception of the durum-derived 20238, this was confirmed in the current study. With three lines in common there was agreement in hardness ranking with results reported by Gazza et al. (2016), but poorer agreement with Hayes et al. (2012) (5 lines in common), particularly for 251b which those authors characterised as medium hard but was found to be soft in the current study. It is noted that the PW lines with highest flour extraction at both sites in 2018 were also higher in kernel weight, while no trend was observed for test weight.

4.3. Rheology

High values for mixograph peak time and integral are interpreted as indicators of high gluten strength (Isaak et al., 2019). The wholemeal mixograph results are suggestive of 235a having good dough quality, and 251b, 20238 and Ot38 all being weaker. The ability to maintain curve width and height over sustained mixing (5.5 min) is also indicative of good dough strength, and this was again seen in 235a. Overall 235a was highly comparable to Wedgetail. This supports the work of Pogna et al. (2014) where this line was found to have significantly higher sedimentation volume, another indicator of gluten quality, compared to other PW lines evaluated. Weaker dough strength is indicated by low peak time and integral, and by high breakdown and descending slope values, as seen in 20238 and Ot38. Within PW lines, protein was not closely related with any mixograph parameters, in line with data presented by Murphy et al. (2009) showing no relationship between protein and PW bakery mixing time.

Flour mixograph data were very closely aligned with those obtained from wholemeal samples, particularly peak time and integral; and bakery mix time trended closely with both mixograph peak time and DoughLAB dough development time. Pogna et al. (2014) found PW lines commonly inherit high molecular weight glutenin subunits (HMW-GS) from the wheat parent; and Hayes et al. (2012) reported encouragingly high unextracted polymeric protein (UPP) contents in a broad range of PW pedigrees. These observations support the expectation that PW lines can possess good dough mixing and breadmaking potential. The PW mixing curves obtained from both the mixograph (Fig. 2) and DoughLAB were characteristic of doughs with significant gluten content, notwithstanding variability in quality. Good agreement was found between both instruments for characterisation of dough strength (e.g., mixograph peak time and integral, and DoughLAB development time and stability) and of weakness (e.g., mixograph breakdown and width at 5.5 min, and DoughLAB softening at 3 min). A negative association was found between DoughLAB water absorption and PSI in PW, which is the opposite of what is observed in common wheat. Within soft wheats, softer kernel texture is associated with lower water absorption due to lower levels of starch damage, smaller flour particle size and lower arabinoxylan content (Kiszonas et al., 2013). High water absorption is particularly influenced by high arabinoxylan content, a component of endosperm cell walls, and has been selectively bred for in hard bread wheats (Delcour and Hoseney, 2010). On the other hand, high water absorption is undesirable in soft wheat flours. PW has not been subjected to the same selection pressure, and it is probable that the soft-grained genotypes in this study have high arabinoxylan contents (thick endosperm cell walls), conferring high water absorption.

4.4. Baking performance

In contrast to the observations of Lachuga et al. (2023) and Murphy et al. (2009), baking performance of the PW lines in this study was not uniformly inferior to the annual wheat control, with 11955 and OK72 producing loaves of very good quality compared to Wedgetail. Baking quality is complex and is determined not only by total grain protein, but also extensively influenced by the composition of wheat protein subunits (Xue et al., 2019; Sharma et al., 2020) with the balance between strength (or elasticity) and extensibility required for best results (Carson and Edwards, 2009). Such balance allows for the retention of CO_2 produced during the fermentation stage without impeding the expansion of gas cells during proofing and baking. Weak (inelastic) doughs are unable to retain gas without rupturing, producing low volume breads featuring coarse open crumb with thick-walled cells. Inextensible doughs impede cell expansion, producing low volume breads with dense, firm crumb. Thus, good baking quality is reflected in good loaf volume but also in appealing crumb structure of small, even cells with fine walls and this was seen in 11955 and particularly OK72, which both outperformed Wedgetail for these traits. The greatest volume and specific volume of all lines was recorded for OK72, while 11955 produced a softer crumb than Wedgetail at the one site it was examined. With rheological parameters indicating greater gluten strength, 235a was expected to have superior baking quality among the PW lines, however, like 251b, internal structure scores were low due to open, thick-walled crumb cell structure, particularly from grain produced at Mandurama in 2018.

Among PW lines, volume and specific volume were found to increase with increasing mixograph peak height, while loaf external appearance improved with greater DoughLAB energy to peak and lower softening. Crumb firmness can be a function of loaf volume because limited gas retention and expansion during baking results in a firmer, less airy crumb structure. However, within PW genotypes, no relationship was observed between volume and firmness indicating crumb structural characteristics, rather than volume, were responsible for texture differences. Line 251b, which was identified as having weak gluten in rheological tests, was the poorest PW baking performer with short bakery mix times and poor external appearance and internal crumb structure scores, particularly from grain produced at Mandurama in 2018. Of the four PW genotypes assessed for baking quality in the present study, only 251b was found by Pogna et al. (2014) to possess HMW-GS that were likely inherited from the wheatgrass parent. Processing of 251b was problematic in the present study with very sticky dough persisting throughout all handling stages, particularly from grain produced at Mandurama in 2018. Bakery water absorption for this line (and 11955) was reduced by 2% which improved handling but still resulted in sticky dough for 251b immediately after mixing.

A negative association was observed between PSI and crumb firmness, with softer grain PW producing softer crumb. While wholemeal colour was a good indicator of flour colour parameters, PW grain colour was found to be a poor predictor of wholemeal colour, especially a* values, in agreement with Adams et al. (2013) who also found a negative correlation between grain and wholemeal redness in annual wheat. PW flour colour was strongly aligned with crumb colour, especially b*. Crumb colour of 235a and 251b were visually described as yellow-brown while 11955 and OK72 were a more appealing creamy-yellow colour. Crust colouration in bread is due to Maillard reactions between sugars and proteins (Murata, 2021), and higher protein content would lead to greater browning as reflected in higher a* for PW compared to Wedgetail.

The strong baking performance of PW lines in this study contrasts with that of Murphy et al. (2009), who concluded that PW baking qualities were inferior to that of annual wheat. In their study, all PW breeding lines tested shared a pedigree similar to 235a and 251b. Recent testing of a greater diversity of PW material among grain industry end-users generally observed high functional qualities among most PW lines tested, with utility to form a range of products (Newell, 2021). For example, PW lines such as Summer 1, 11955, OK72 and even 235a demonstrated that they could produce a high-quality product using artisan baking methods, rating well for external and internal loaf characteristics (Sinclair et al., 2021). The favourable baking properties observed in the present study suggests that there is substantial opportunity for selection of PW lines with desirable flour attributes, for both artisan and general baking applications alike.

5. Conclusion

The variation in grain parameters, flour milling yield, dough rheology and breadmaking quality was anticipated. However, there were unexpected combinations of traits which would suggest caution in applying traditional assumptions based on annual wheat. Lines 11955 and OK72, despite classification as extra soft and soft respectively, displayed higher water absorption than harder grained lines. Furthermore, although rheological tests indicated moderate gluten strength only, these lines exhibited better baking performance than the line 235a which displayed good gluten strength more comparable to Wedgetail wheat. Although these findings are based on a small sample set, they nevertheless indicate that general expectations and predictive tests used for common wheat do not necessarily translate to PW. This paper has investigated baking performance with very encouraging results and further studies utilising PW in other products is warranted. With refinement of formulations and conditions to maximise performance, PW can clearly offer good functionality for food applications given the gluten and starch properties observed in this study. Further assessments of PW lines would provide an opportunity to apply selective breeding to this crop to produce lines combining both longevity, higher grain yield and food functionality.

Author contributions

Denise Pleming: Conceptualisation, Methodology, Data curation, Writing -Original draft. **Matthew Newell:** Conceptualisation, Methodology, Formal analysis, Writing Reviewing & editing. **Richard Hayes:** Conceptualisation, Methodology Formal analysis, Writing Reviewing & editing. **Ke Hong Tang:** Conceptualisation, Methodology, Writing Reviewing & editing. **Beth Penrose:** Conceptualisation, Methodology, Data curation, Formal analysis, Writing Reviewing & editing. **Matthew Wilson:** Conceptualisation, Methodology, Writing Reviewing & editing. **Annie Riaz:** Conceptualisation, Methodology, Writing Reviewing & editing. **Chris Blanchard.** Conceptualisation, Methodology.

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Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

Data will be made available on request.

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