Article

Genotype by Environment Effects on Durum Wheat Quality and Yield-Implications for Breeding

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ABSTRACT

The main focus of a durum breeding program is to create high yielding, adapted durum wheat varieties that meet grain quality standards. Using modern statistical approaches we show how comprehensive data sets can be used to readily identify high performing and stable genotypes. A subset of 12 durum wheat genotypes were selected from breeding trials grown in five different locations in New South Wales, Australia, over three seasons constituting 15 dryland environments. Grain yield and quality traits were determined and for a subset of six genotypes, pasta quality traits were assessed. For non-pasta traits and yield, genotype, year, location and their interactions were statistically significant. Plots of overall performance versus stability allowed identification of the best genotypes for each trait with clear improvements noted in the more recent breeding material compared to older varieties. This analysis indicated high heritability for traits such as colour, dough strength, yield and milling potential. Using this approach, breeders can easily identify high performing genotypes for release or as parents in crossing.

KEYWORDS: durum wheat; heritability; breeding; genetics; grain quality

ABBREVIATIONS

G × E, genotype by environment; GY, grain yield; TW, test weight; TGW, 1000 kernel weight; HVK, hard vitreous kernels; GP, grain protein; SKHI, single kernel hardness index; MY, total mill extraction; SY, semolina mill extraction (break + semolina); Semo, semolina only extraction; *L**, brightness; *a**, redness; *b**, yellowness; WI, whiteness index; BI, browning index; MPT, mixograph peak dough development time; RBD, mixograph resistance breakdown; WG, wet gluten; GI, gluten index; OCT, pasta optimum cooking time; DPL, uncooked pasta brightness; DPa, uncooked pasta redness; DPb, uncooked pasta yellowness; DPWI, uncooked pasta whiteness; CPL, cooked pasta brightness; CPa, cooked pasta redness; CPb,

cooked pasta yellowness; CPWI, cooked pasta whiteness; CSTAB, cooked pasta colour stability; CL, Cooking loss; WABS, pasta water absorption; Firmness, Firmness peak height; SPH, stickiness peak height; SArea1, stickiness peak area; ME-LMM, multi-environment linear mixed model; BLUEs, best linear unbiased estimates; FAST, Factor Analytic Selection Tools

INTRODUCTION

Durum wheat (*Triticum durum* Desf.) is an important crop for the human diet and while durum wheat only accounts for 5–8% of world wheat production, it is important in producing a range of food products, such as semolina, pasta, burghul wheat, couscous and desserts unique to durum [1]. The main durum-growing regions are the Middle East, Southern Europe, North Africa, the former Soviet Union, North America, Mexico, India and some minor but important production areas like Argentina and Australia [2]. Most of the durum wheat is grown under rainfed conditions in semiarid regions, typically characterised by unpredictable and highly variable seasonal rainfall impacting on yield and quality stability [3]. In the Australian environment, most durum wheat is grown in north eastern New South Wales and South Australia and these regions represent variable soil and climatic conditions. This is particularly during the grain filling period, where water and nitrogen variability and heat stress can occur and collectively these may improve or deteriorate durum wheat processing quality [4,5]. Obtaining genetic increases in grain yield and quality to meet market requirements is a challenge in a highly variable environment like Australia. This results in slow genetic advance in breeding because genetic variation in yield and quality is influenced by genotype x environment (location/year) and their interaction and this is managed in breeding by conducting field trials in replicated plots in multiple locations representative of the main production zones. The idea is to build "a picture" of a genotypes performance against reference varieties over multiple seasons that allows the breeder to select desirable genotypes for improvements in one or more characters associated with yield, quality, adaptation or disease resistance considered necessary in new varieties for release.

It is important to determine the effects of genotype (G), environment (E) and their interaction (G × E), i.e., variation in genotype response under different environmental conditions on durum wheat quality under rainfed conditions but this has never been reported in the Australian environment although one study has reported these effects for irrigated durum wheat [5]. Several G × E studies have been reported in rainfed environments for durum wheat yield in other countries with fewer studies covering durum quality [3,6–11]. Genotype-environment interactions are important in evaluating cultivar adaptation, selecting parents and developing improved genotypes. If the ranking of genotypes differs between environments this makes it more difficult to identify superior

breeding genotypes since the measured trait values are affected more by environmental variation than genetic differences. If G × E is large, then testing in multiple environments is required to assess cultivar performance accurately. An ideal stable genotype is one that performs for agronomic and quality across a wide range of environments showing good performance regardless of variation in environmental conditions. Such varieties are more acceptable in the market because they provide more processing stability in milling and pasta making [8]. Quality traits that are less affected by E and more by G make genetic gain more effective. Identifying such quality traits is important in the context of the Australian environment and genotypes grown in order to advance through breeding.

Historically, G × E analyses of multi-environment data was undertaken using simple regression approaches [12,13]. There has also been a strong focus on using extensions of analysis of variance through additive main and multiplicative interaction (AMMI) approaches [14,15]. Unfortunately, these methods lack flexibility for more complex unbalanced multi-environment data where multiple sources of heterogeneous non-genetic variation are potentially present in individual environments [16,17]. More flexible modern G × E analysis approaches are available through the adoption of highly structured multi-environment linear mixed models [18]. In particular, Smith et al. [18,19] justify the use of a Factor Analytic model to parsimoniously model the G × E interaction effects. These models can then provide a basis for conducting variety stability analysis through approaches such as Factor Analytic Selection Tools (FAST) [20].

Our objectives were to (i) understand G, E and G × E in the northern Australian environment for durum wheat yield and quality (ii) to use that knowledge as a platform for future selection for quality traits and design more effective selection strategies.

MATERIALS AND METHODS

Plant Material

The multi-trial data consisted of four registered durum wheat varieties (EGA Bellaroi, Caparoi, Hyperno and Jandaroi) and eight advanced experimental genotypes, three of which have recently been commercially released: DBA Aurora, released in 2014, DBA Lillaroi released in 2015 and DBA Vittaroi released in 2017 (**Table 1**). These genotypes were a subset from the larger advanced stage four trials conducted by the Northern Program of Durum Breeding Australia (a joint project between New South Wales Department of Primary Industry, The University of Adelaide and the Grains Research and Development Corporation). The yield data were analysed for the entire trials. Quality data were generated only for the above selected genotypes because of the resources needed to analyse all the genotypes in the trials. The 2013 trial at Edgeroi was not included in the analysis of grain yield (but was for quality traits) because of uneven germination and establishment due to poor soil moisture at planting.

Table 1. Pedigree, origin and maturity of durum genotypes included in the study.

Genotype	Pedigree	Year of release	Days to 50% heading
240578	960707/980947	Breeding line	126
280012	200325/200468	Breeding line	126
280115	200325/200468	Breeding line	126
290491	230616/230800	Breeding line	126
290564	230616/230800	Breeding line	127
DBA Lillaroi	960273/980596	2015	125
DBA Vittaroi	200856/980990	2017	126
Caparoi	LY2.6.3/930054	2008	128
EGA Bellaroi	69778/870015//SRN/SULA	2003	127
Jandaroi	(Souri/Wollaroi)/Kronos	2007	123
DBA Aurora	Tamaroi*2/Kalka//RH920318/Kalka///Kalka*2/Tamaroi	2014	127
Hyperno	Kalka "S"/Tamaroi	2009	126

Agronomic Details

Field trials were conducted at 5 locations in New South Wales, Australia (**Table 2**) over 3 seasons (2012, 2013 and 2015) with each trial randomised as row column designs using DiGGer [21] with three replicates. We avoided 2014 because several sites suffered weather conditions that led to poor grain vitreous levels which can interfere with some quality traits. These types of studies need to be representative of the long-term average for the environments chosen in evaluating the usefulness of G × E studies. A constant limitation to all G × E studies of quality traits is the cost and may explain why most studies are for an average of 2.5 ± 1.8 years [22].

The trials were planted with plot lengths of 10 m or 8 m (the plot lengths varied to suit the controlled traffic set up of the grower co-operators). The plots were trimmed by 1m on each end and the actual plot length was measured at maturity to calculate yield in t/ha. Details of the soil type, pH and applied nitrogen is given in **Table 2**. Starane (Fluroxypyr) or Tordon 242 (MCPA + Picloram), both Group I herbicides, were applied to control broadleaf weeds prior to growth stage 30. N management consisted of determining the deficit between available soil N and the amount needed to achieve an average grain yield at 13% grain protein. Fertilisers consisted of urea and Granulock Z15 (Incitec Pivot, Southbank, Australia) applied at sowing. The latter was applied at 50 kg/ha which provided 5.5 kg N, 10.9 kg P, 2 kg S and 0.5 kg Zn.

Table 2. Details of trial sites, agronomic data and sowing dates.

Location	Year	Trial sites						
		Breeza	**Edgeroi**	**Narrabri**	**Moree**	**Tulloona**	**North Star**	**Tamworth**
Latitude		31.25 °S	30.11 °S	30.34 °S	29.46 °S	30.33 °S	28.93 °S	31.09 °S
Longitude		150.46 °E	149.79 °E	149.76 °E	149.8 °E	149.78 °E	150.39 °E	150.93 °E
Altitude (m)		295	243	212	212	212	419	404
Soil Classification		Grey cracking clay	Grey cracking clay	Grey cracking clay	Grey cracking clay	Grey cracking clay	Variable	Variable
pH (CaCl$_2$)	2012	nd	nd	nd	nd	nd	nd	nd
	2013	8.1	7.4	nd	7.0	nd	7.6	7.7
	2015	8.0	8.0	7.1	7.8	7.8	7.4	7.2
Total applied N (kg/ha)	2012 [A]	47.25	47.25		47.25		47.25	47.25
	2013	241	52		60		56	67
	2015	33	5.5	5.5	5.5	106	16	9
Sowing dates	2012	23/05/2012	17/05/2012		16/05/2012		15/05/2012	25/05/2012
	2013	21/05/2013	10/05/2013		9/05/2013		7/05/2013	27/05/2013
	2015	8/05/2015	29/06/2015	21/05/2015		23/05/2015	13/05/2015	8/06/2015

[A] = no N management; nd = no data available.

Technological Tests

Following harvest, grain analyses were performed on cleaned samples (Carter Dockage Tester, Simon-Carter Company, Minneapolis, USA). The following quality traits were evaluated with details described previously [5].

Grain tests

Test weight (TW) is a measure of grain density and how well the grain packs into a given volume with values of 76 kg/hL or higher desirable. The weight of 1000 kernels (TGW) is an indicator of grain size and a rough predictor of milling performance and is related to TW. The percentage of hard vitreous kernels (HVK) was determined using a farinator to cut 6 sets of 50 kernels, taking an image with a smart phone and examining the image on a PC. Scoring for vitreous and non-vitreous was performed visually by a trained operator, to provide consistency in the arbitrary measurement. Vitreous grains appear translucent, while starchy grains appear opaque with small white spots of varying size (piebald) or completely white. Single kernel hardness index (SKHI), or grain hardness was measured using NIR with a calibration developed from the single-kernel characterisation system [23]. Grain protein and moisture were measured using in-house calibrations on an NIRSystems model 6500 spectrophotometer (Foss NIRSystems Inc., Laurel, MD, USA) as outlined previously [23].

Semolina tests

Grain was milled into semolina by tempering the grains for 18 h to 15.0% moisture and milled in an experimental mill (model MLU 202; Buhler, Uzwill, Switzerland) equipped with three break rolls and two reduction rolls [24] (Approved Method 26-41). Three traits were measured: If TPW = total products weight (bran, pollard, break, reduction and semolina fractions) then total mill extraction % (MY = total flour weight/TPW × 100); semolina mill extraction % (SY = semolina weight (three break fractions + semolina)/TPW × 100); semolina only extraction (SO = semolina weight/TPW × 100), so differs from SY in not having the three break fractions included. Semolina colour was evaluated by measuring L^* (brightness, 100 = white, 0 = black), a^* (+ve is redness. −ve is greenness), b^* (+ve is yellow, −ve is blue), and whiteness index (WI) traits by means of a Minolta Chroma Meter CR-410 (Biolab Australia, Sydney) in triplicate. A browning index (BI) is also measured where BI = 100 − L^*.

Dough tests

Dough quality was measured using two methods, mixograph and gluten index because there is no single best estimate of dough properties of durum [25]. Mixograph (duplicate determination per sample) was conducted with a 10-g mixograph (National Manufacturing Co., Lincoln, NE, USA). MixSmart software supplied with the mixograph was used to determine mixograph peak dough development time (MPT) and resistance breakdown (RBD) defined as: 100 × ((width of the curve at peak mixing time (WAP) minus width of the curve after 8 min mixing, (W8)/WAP). Gluten index (GI) was assessed on semolina samples [24] according to AACC Approved Method 38-12 using a Glutomatic 2200 (Perten Instruments, Huddinge, Sweden). GI was expressed as the ratio of the wet gluten (WG) remaining on the sieve after centrifugation to the total wet gluten weight.

Pasta tests

To obtain sufficient semolina to make pasta on a 1 kg scale, we pooled field replicate samples. Semolina was purified on a locally made, small-scale purifier and this was used to prepare long pasta (spaghetti) with a Namad pasta extruder which was then dried at 50 °C for 13 h and cooled to 25 °C under controlled humidity conditions [5]. All pasta samples were cooked to their optimum cooking time (OCT), which is the time taken for the pasta starch central core to disappear. Then texture testing was performed on cooked pasta which was assessed for firmness peak height and area (F-Area) and stickiness as peak height (SPH) and two areas under the curve (SArea1). In addition, water absorption (WABS) and cooking loss (CL), were measured as described previously [5]. Uncooked spaghetti colour was measured on the HunterLab scale for L^*, a^*, b^* and whiteness index (WI) in triplicate using a Minolta camera CR-410 (Konica Minolta Sensing Inc. Osaka, Japan). Cooked pasta colour was measured on optimally cooked pasta. Colour stability was determined as described previously [5].

Statistical Analyses

Multi-environment linear mixed model

Each of the pasta and non-pasta traits was initially analysed using a multi-environment linear mixed model (ME-LMM) that partitioned and accounted for all sources of genetic and non-genetic sources of variation. Let $\boldsymbol{y} = (\boldsymbol{y}_1^T \ldots \boldsymbol{y}_t^T)^T$ be the

vector of observed responses across t environments (Locations × Years), then the LMM had the form

$$y = X\tau + Z_e u_e + \epsilon \tag{1}$$

where $X\tau$ is the fixed component of the model with explanatory matrix X and vector of fixed effects τ that were conformably partitioned to contain main effects for factors Year, Location and Genotype, two-way interaction effects for each pairwise combination of the factors and three-way interaction effects for all factors combined. The $Z_e u_e$ was a random component of the model where Z_e is an indicator matrix and u_e are random effects conformably partitioned to contain effects to adequately account for differences between field reps in each environment as well as effects for differences between milling days for milling based traits. To provide necessary flexibility to the ME-LMM, the residual model ϵ was appropriately partitioned to $\epsilon = [\epsilon_1^T \dots \epsilon_t^T]^T$ where the residuals within the jth environment were assumed to be distributed $\epsilon \sim N(\mathbf{0}, \sigma_j^2 I)$. Identification of residual outliers was determined using the alternative outlier model (AOM) approach derived in [26] and outliers were then down-weighted through the inclusion of separate indicator random covariates in (1).

From the fitted ME-LMM, the Wald statistics analysis of variance table was calculated for the complete hierarchy of fixed effects and summarized with degrees of freedom for each of the effects. Additionally, the best linear unbiased estimates (BLUEs) of the Year × Location by Genotype effects were extracted from the ME-LMM and summarised for relative performance across the environments by subtracting the environment mean from the within environment Genotype BLUEs.

Heritability

To accurately determine trait heritabilities at each of the environments the non-pasta traits were analysed using an alternative multi-environment LMM of the form

$$y = X^*\tau^* + Z_e u_e + Z_g g + \epsilon \tag{2}$$

where terms differing from (1) include a reduced fixed component $X^*\tau^*$ containing the explanatory matrix X^* and vector of fixed effects τ^* conformably partitioned as main effects for factors Year and Location and their two-way interaction effects. The term $Z_g g$ was then a random component with indicator matrix Z_g and vector of effects g representing a set of multiplicative Location × Year ×

Genotype random genetic effects. The effects are assumed to be distribution $g \sim N(\mathbf{0}, \mathbf{D} \otimes \mathbf{I})$ where $\mathbf{D}$ is a $t \times t$ diagonal matrix with diagonal elements $(\phi_1^2, \ldots, \phi_t^2)$ representing the t genetic variances at each of the environments. Heritability within the jth environment is then calculated using the formula from [27], namely

$$H_j^2 = 1 - \frac{PEV_a(\widetilde{\boldsymbol{g}}_j, \widetilde{\boldsymbol{g}}_j)}{2\phi_j^2} \qquad (3)$$

where $PEV_a(\widetilde{\boldsymbol{g}}_j, \widetilde{\boldsymbol{g}}_j)$ is the average pairwise prediction error variance of the best linear unbiased predictors (BLUPs) of $\boldsymbol{g}_j$ and ϕ_j^2 is the genetic variance associated with the jth environment.

Stability analysis

For non-pasta traits, the assessment of genotype performance and stability was determined using the FAST approach of Smith and Cullis [20] This required each of the traits to be analysed using an extension of the ME-LMM defined in (2). This extension involved a specification of the multiplicative Location × Year × Genotype random genetic effects to have distribution $g \sim N(\mathbf{0}, \boldsymbol{\Delta} \otimes \mathbf{I})$ where $\boldsymbol{\Delta}$ is parameterized as an $t \times t$ unstructured covariance matrix with diagonal elements that reflect the genetic variation within each of the environments and off-diagonal elements that reflect the genetic relationships between each pair of environments [18]. As computational estimation of $\boldsymbol{\Delta}$ is difficult, a parsimonious approximation was sought by defining the genetic effects using a Factor Analytic (FA$_k$) model [18,19]. Under this approximation, the genetic effect for line i in environment j becomes

$$\begin{aligned} g_{ij} &= \lambda_{j1} f_{i1} + \lambda_{j2} f_{i2} + \cdots + \lambda_{jk} f_{ik} + \psi_{ij} \\ &= \lambda_{j1} f_{i1} + e_{ij} \end{aligned} \qquad (4)$$

where f_{ir} is the rth hypothetical factor, $r = (1, \ldots, k)$, and λ_{jr} is the associated rotated loading in environment j, $j = 1, \ldots, t$ with environment specific genetic residual ψ_{ij}. To aid in interpretation of the approximate genetic effects Smith and Cullis [20] developed Factor Analytic Selection Tools or a FAST approach for to determining overall performance (OP) and stability (ST) of varieties across the environments. From the fitted multi-environment model the OP and ST for line i was calculated using

$$OP_i = \frac{1}{t}\sum_{j=1}^{t} \tilde{\lambda}_{1j} \tilde{f}_{i1} \qquad\qquad ST_i = \sqrt{\frac{1}{t}\sum_{j=1}^{t} \tilde{e}_{ij}^2} \qquad (5)$$

Similar to principal component analysis, the OP uses a function of the first component of the FA_k model to determine average overall performance of a variety across environments. The ST then uses the remaining $k-1$ components of the FA_k model to provide a numerical quantification of the stability of the variety across environments with lower values indicating greater stability.

Computational

All statistical models were computationally conducted using the flexible linear mixed modelling R package ASReml-R V4 [28] and downloadable from https://www.vsni.co.uk/software/asreml-r. Model diagnostics were assessed using the R package ASExtras4 available from https://mmade.org/asextras4/.

RESULTS

Phenotypic Trait Summaries

A phenotypic boxplot of the 18 traits shows the distribution in trait values across the 15 environments (**Supplementary Figure S1**) which provides a link between overall genotype performance to environmental differences. The length of each boxplot is a reflection of the phenotypic variability found for all the genotypes in each environment. A wide spread in the distribution of the boxes reflects a stronger influence by environment (Y × L) than a narrow distribution of boxes. GY tended to range 3–5 t/ha but two environments (2013-Tamworth and 2012-Tamworth) were associated with much lower yield than others while 2015-Narrabri and Breeza stood out with the highest yield. Generally, the different years tended to cluster except for 2013-Tamworth and 2012-Tamworth. Possible reasons for this variation in yield are relatively poor soil conditions together with low rainfall during August–October in 2012 and 2013 as shown in the histograms. 2012 was the driest season for these months in contrast to 2015 which had significant rain in August. The August - September period coincides with stem elongation, flag leaf and ear emergence which are critical stages for yield formation. Grain filling occurs from mid/late September to the end of October. Good moisture conditions at these critical growth stages determine the grain size and grain yield (**Supplementary Figure S5**). The genotypes showed excellent TW values, mostly >80 kg/hL and reaching very high values >85 kg/hL. Values for TGW were high (>47 g) at two sites while 2013-Tamworth had low values (for durum) with the median

<35 g, probably a reflection of stress conditions at this site, as noted above, although this was not the situation at 2012-Tamworth. In most environments HVK > 75 which would ensure sound grain while at 2015-Tamworth and 2015-Narrabri, lower median values were achieved which would lead to a feed grade in the Australian grain receival standards. Generally, GP exceeded 12% (11% mb) which is important to achieve good overall performance except at 2015-Narrabri where the median protein was <11% which also had one of the highest GY. GY is negatively correlated to GP but 2013-Tamworth with even lower GY had median GP ~13%. While grain hardness varied ~75–95 SKHI it was not associated with SY and Semo. Both SY and Semo are closely related and their distributions show a lot of similarity except in 2012 (**Supplementary Figure S1**). Colour traits (L^*, a^*, b^*, WI, BI) seem to show less variation across environments, especially for L^* and a^*. Dough properties represented by the MPT, RBD and GI plots show a quite even and narrow spread for MPT around 3–5 min and more variation in distribution for RBD and GI with most values >60. These boxplots do not provide information about the G × E interaction (see below).

Effect of Genotype, Environment and Their Interaction on Grain Yield, Grain and Semolina Quality Traits

In this study we have used location and year as environment. For grain yield and 16 quality traits, model (1) was fitted and showed that genotype (G), environment (L and Y) and genotype by environment (Y × G, L × G, Y × L × G) were significant for most grain, semolina and dough quality traits (**Table 3**). The extent of the effect varied as shown by the magnitude of the Wald statistic. Compared to Y and L, the statistics indicate there were strong G effects for TW, TGW, GP, a^*, b^*, WI, WG and GI. Whereas, environmental effects (Y and L) were greater for traits GY, HVK, SKHI, MY, SY. That all traits except L^*, MPT and RBD had highly significant Y × L × G Wald statistics indicates multi environment trials are needed to evaluate most of these traits. For milling traits, while MY and SY had lower Wald statistics for G compared to Y and L, for Semo trait, the Wald statistics were higher for G than L but not Y. This might suggest better selection is possible using this trait. Heritability estimates (see below) suggest good genetic gain should be possible for MY, SY, Semo, GI, b^*, a^*, WI with other traits showing a lot more variability in heritability across environments.

Table 3. ANOVA Table of Wald statistics of fixed terms in each of the trait models for non-pasta traits. *P*-value significance of terms is represented by superscript stars (*** <0.001; ** <0.01; * <0.05). Bolded numerics in each column represent the highest order significant terms respecting marginality. Phenotypic trait minimums, maximums and averages are given at the bottom of the table.

Effect	GY	TW	TGW	HVK	GP	SKHI	MY	SY	Semo	S-*L* *	S-*a* *	S-*b* *	WI	MPT	RBD	WG	GI
Y	10313***	376.0***	36.50***	273.7***	116.4***	282.4***	1610***	753.6***	3044***	35.63***	183.4***	197.2***	195.2***	680.1***	248.2***	306.0***	285.5***
L	6760***	225.0***	370.7***	617.4***	247.1***	99.14***	869.1***	629.4***	616.4***	318.9***	186.0***	600.8***	578.6***	234.0***	86.46***	197.9***	119.8***
G	1863***	1374***	1027***	98.89***	809.7***	99.12***	660.7***	598.6***	2423***	255.7***	1418***	11238***	11090***	254.0***	190.1***	706.1***	1014***
Y × L	1213.0***	284.3***	208.7***	57.22***	90.64***	334.7***	108.3***	77.55***	129.8***	**14.97***	31.32***	64.11***	52.40***	**36.40***	**50.60***	81.16***	105.5***
Y × G	139.0***	186.0***	119.7***	128.3***	153.2***	166.0***	21.43	29.89	85.19***	31.32	28.18	107.1***	119.0***	**76.47***	**76.43***	33.92*	125.4***
L × G	1960***	336.7***	366.4***	130.8***	177.4***	235.8***	147.3***	137.7***	600.3***	**84.64***	**89.95***	355.4***	353.1***	**127.2***	82.27	214.4***	263.4***
Y × L × G	**215.0***	**172.3***	**122.6***	**177.7***	**131.2***	**179.3***	**107.7***	**129.7***	**386.7***	65.14	**98.70***	**306.0***	**281.8***	48.22	61.82	**131.8***	**233.5***
Min	1.18	77.9	33.8	21.4	9.2	71.3	76.4	68.4	50.0	80.3	0.2	25.0	−64.0	2.21	7.14	16.7	41.3
Max	6.01	86.7	54.7	99.3	17.4	96.5	82.2	73.6	63.5	84.7	0.29	36.2	−31.6	8.23	95.7	40.2	98.8
Mean	4.03	82.5	42.4	82.0	13.0	87.8	80.0	71.5	57.2	82.2	0.24	31.2	−49.7	3.62	56.5	27.9	79.9

By expressing the best linear unbiased estimates (BLUEs) for each trait as deviations around the environment means (site x year) for all genotypes, it becomes very obvious which varieties show better than average performance and which are worse than average. Selected key traits are plotted in **Figure 1**. Where the desirable directions for traits is positive (GY, GP, WG, HVK, TW, TGW, SY, SEMO, *L**, *b**, MPT, GI) mean performances represented as blue bars while orange bars reflect a less desirable direction and where the magnitude of these responses is shown as the size of the bar. Note for RBD a lower value (orange bar) is desirable. The highest yielding genotypes consistently across the 15 environments were DBA Aurora and Hyperno and the lowest, EGA Bellaroi and Jandaroi. Other genotypes showed variable yield responses or very close to the environment mean (bar with little height) so are not outstanding for yield but whether they are retained depends on other grain quality attributes. For example, DBA Lillaroi while slightly above or equal to the mean yield (9/14 environments) and below for 5 environments, was outstanding for TGW, SY and Semo (**Figure 1** and **Supplementary Figure S2**).
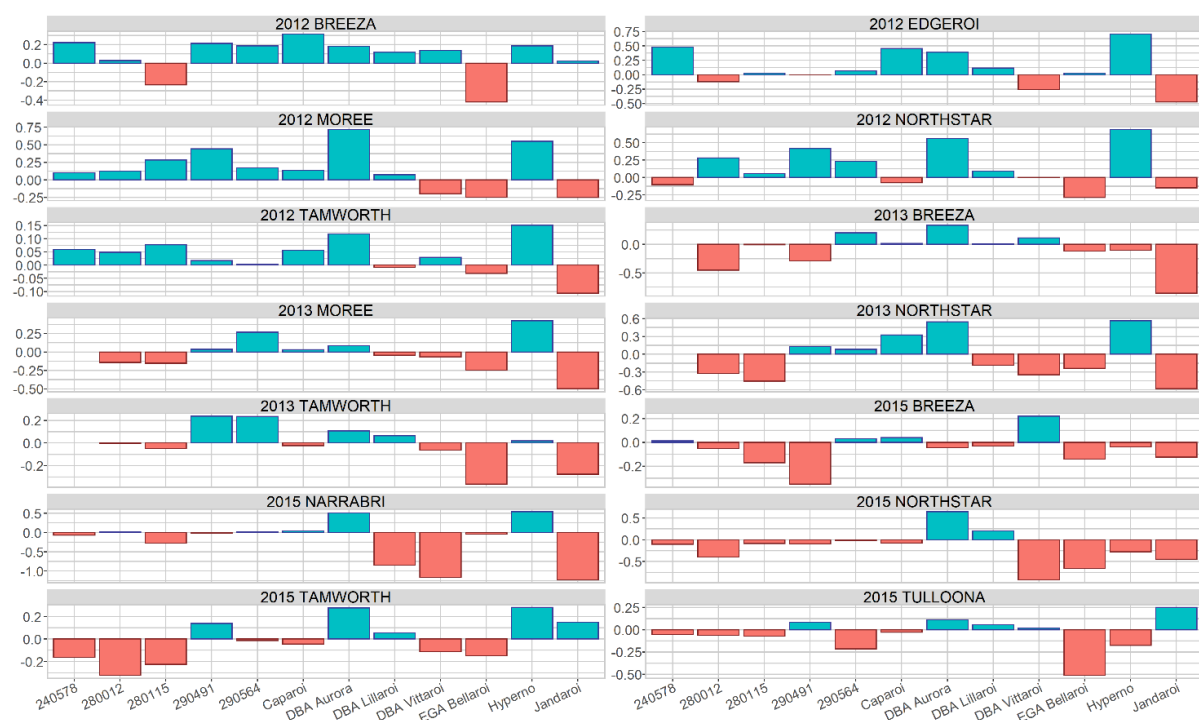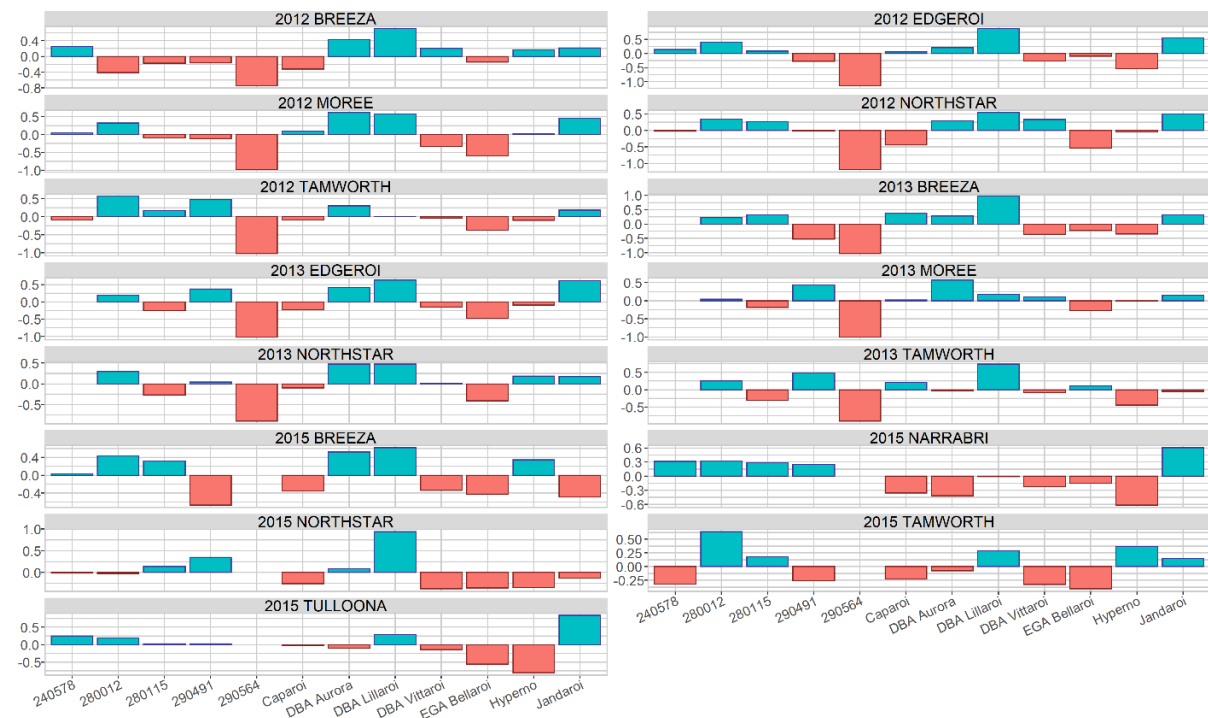

GY

Figure 1. Plots showing the best linear unbiased estimator (BLUEs) values expressed as deviations around the environment (site x year) mean of 12 durum wheats across 15 environments for non-pasta traits. For any given trait, muted blue bars indicate better than average performance of a variety and muted red bars indicates worse than average performance.
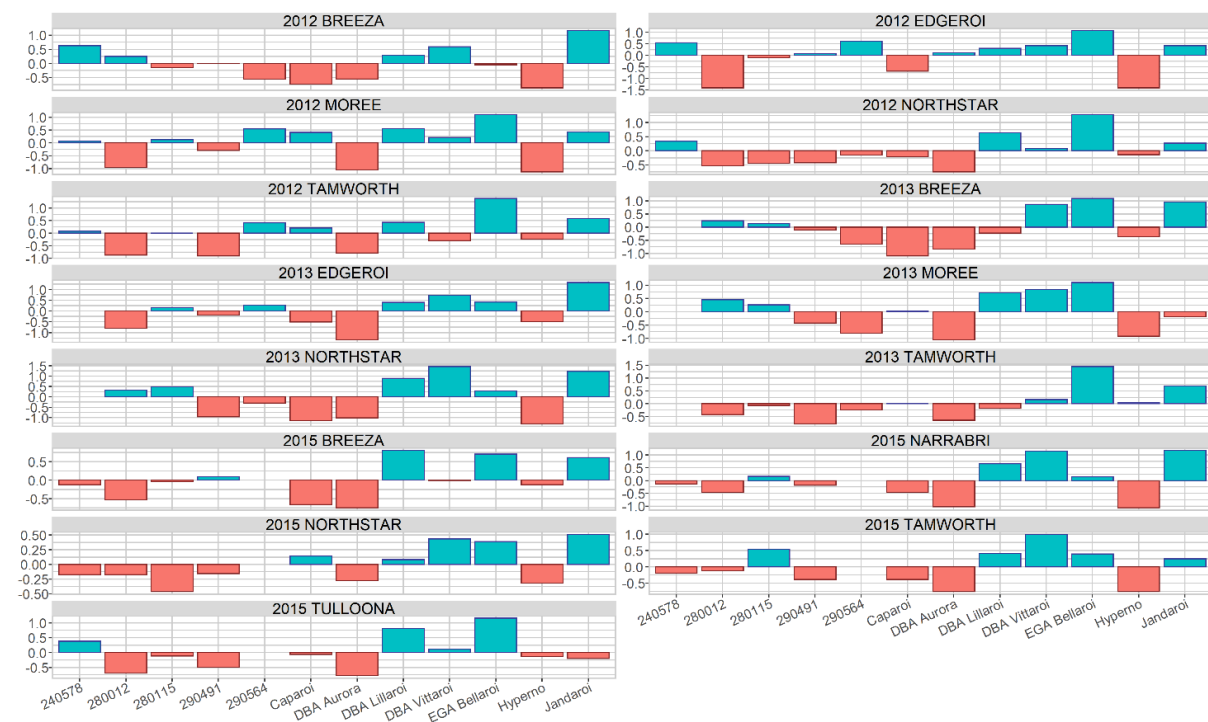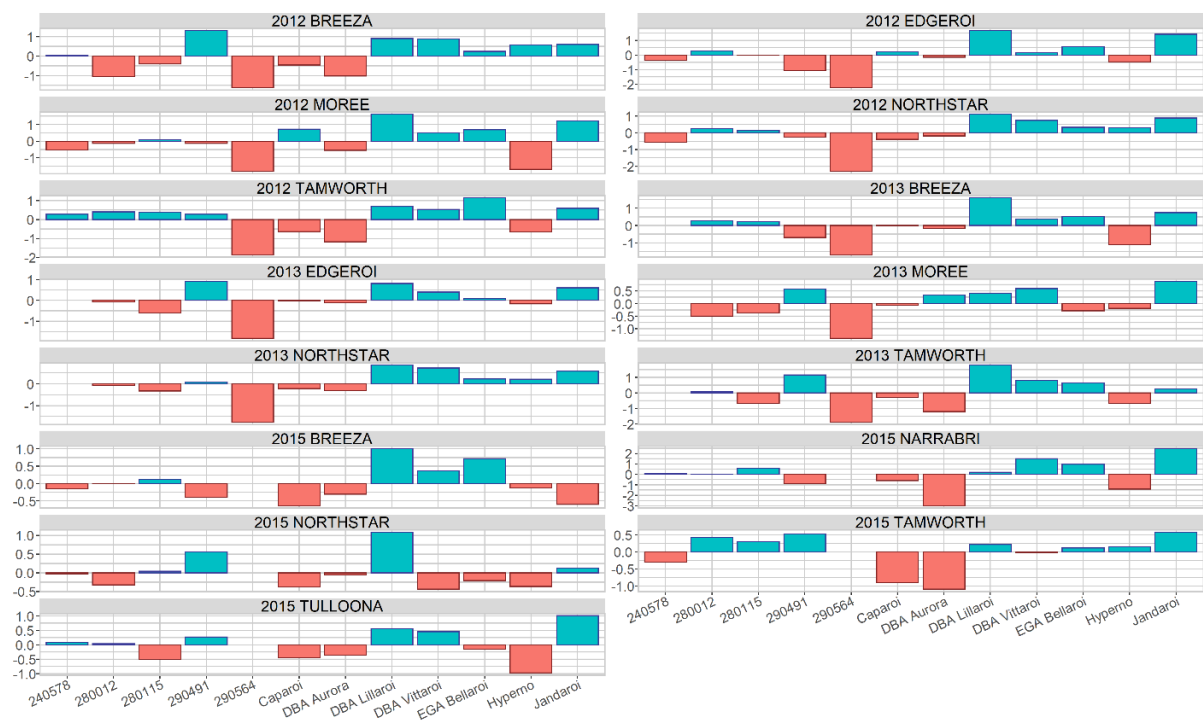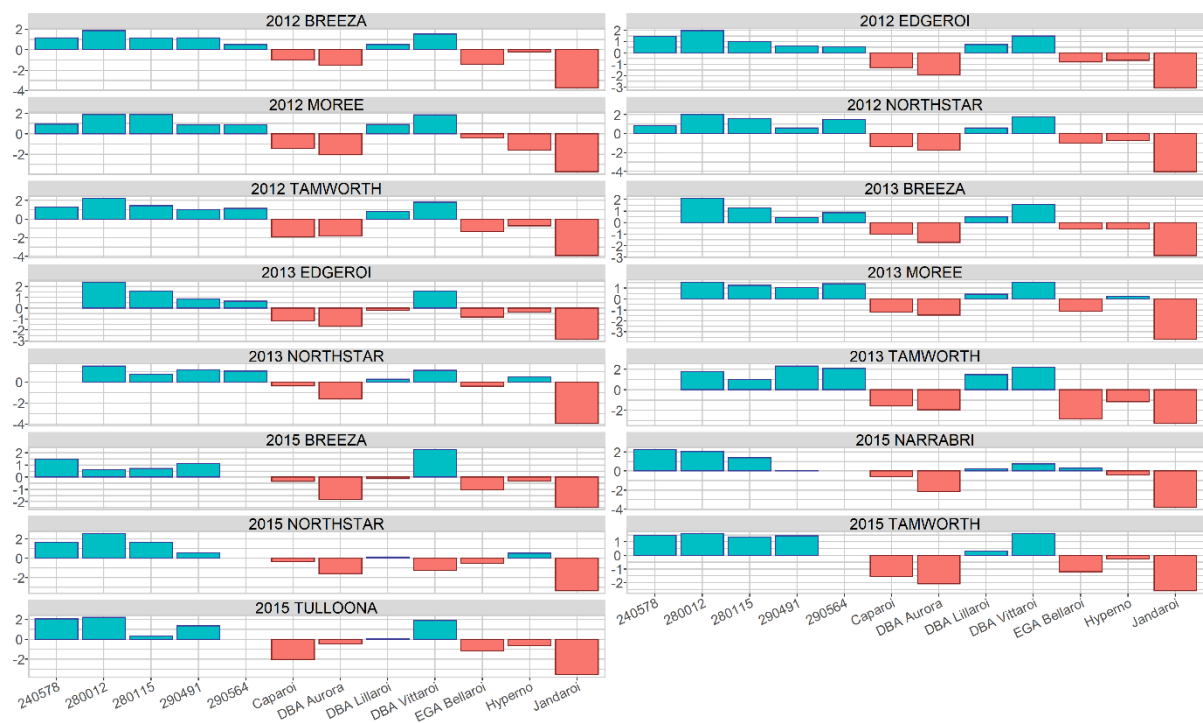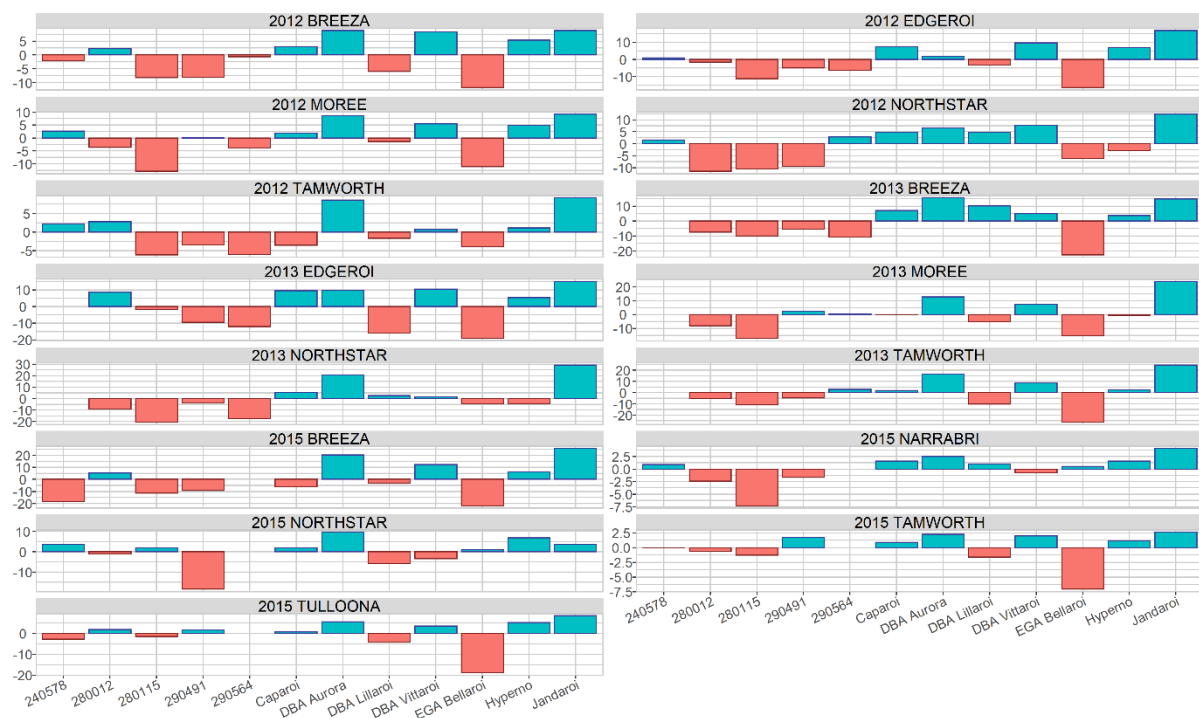
GP

SY

Figure 1. *Cont.*

Semo

S-*b**

Figure 1. *Cont.*

GI

Figure 1. *Cont.*

Inspecting the grain protein responses seems to rank the genotypes as the inverse of yield with the most consistently higher GP performers being EGA Bellaroi, Jandaroi, DBA Vittaroi and DBA Lillaroi and the lowest, DBA Aurora, Hyperno, Caparoi, 290491 and 280012. This is related to high protein grain having less starch (see discussion). The other genotypes were around average or below for yield and protein with 280012 having both below average yield and grain protein so would clearly be undesirable. WG followed a very similar trend to GP for the genotypes across environments (**Supplementary Figure S1**). There was less correspondence between GP and WG for DBA Vittaroi which gave higher than average GP but not for WG while 290564 tended to be above site mean for WG and less for GP, indicating that it is important to measure both although in grain receival specifications, only grain protein is considered in Australia. While the main functional protein is gluten, farmers are paid on grain protein content. Achieving premium grade (DR1) requires a minimum of 13% protein in Australia hence the emphasis on having good genetic potential to achieve this level of protein in the grain.

Average HVK achieved was 82% and genotypes giving consistently lower HVK than average were DBA Lillaroi (9/15 sites), DBA Aurora (13/15) and Hyperno (7/15 sites) while those above site mean were DBA Vittaroi (11/15) and 280012 (14/15)

and 280115 (12/15) (**Supplementary Figure S1**). Genotypes with consistently well above average TGW across environments in order were DBA Lillaroi (14/15 sites), 240578 (10/10) and Jandaroi (13/15) with DBA Lillaroi showing the greatest deviation from site means (**Supplementary Figure S1**). Those below site mean for TGW, in order of frequency and magnitude were Hyperno (13/15), 280115 (14/15), EGA Bellaroi (9/15), 280012 (13/15) and 290491 (10/15). These rankings were not the same for TW despite a significant correlation between TW and TGW (r = 0.40, p < 0.001, **Supplementary Figure S4**), with Caparoi showing higher TW more consistently. For milling quality two traits were measured, SY and Semo with poor performing genotype 290564 consistently below site average (10/10 sites) for both traits (**Figure 1**) For SY, DBA Lillaroi (13/15), Jandaroi (12/15) and 280012 (12/15) gave consistently superior milling performance to the other genotypes while for Semo, 280012 did not perform as well as DBA Lillaroi and Jandaroi with the greatest response from DBA Lillaroi (**Figure 1**). There were low correlations between both TW and TGW with SY and Semo (**Supplementary Figure S4**). Semo seems to be more discriminating of the two measures of milling yield separating the variation between genotypes better than SY. The lowest SY and Semo genotype was consistently 290564 although the TGW and TW data did not point to its low milling potential.

Brightness is impacted by higher protein negatively and is affected more by E than G (**Table 3**). This was clear for $L*$ showing the genotypes with below average GP had higher semolina $L*$ while EGA Bellaroi (higher GP) was below the site mean at 13/15 sites (**Supplementary Figure S2** and **Figure 1**). Jandaroi performed consistently below the site average with the lowest $b*$ and less so for DBA Aurora, Caparoi and to a lesser extent EGA Bellaroi (**Figure 1**). All the other genotypes are more recent in the breeding program than the commercial varieties (EGA Bellaroi, Caparoi, DBA Aurora, Hyperno and Jandaroi) and would be expected to have superior $b*$ due to continuous selection for higher semolina $b*$ in the program as shown with the best being 280012 and DBA Vittaroi. Of note is a lot less variability between environments for 280012, 280115, DBA Vittaroi and 290491 and above average $b*$ is achieved in nearly all of these lines.

Dough properties were assessed by mixograph (peak mixing time and resistance breakdown or dough stability), with longer MPT and lower RBD associated with stronger dough and gluten index, where values >70 are considered strong and >90 very strong dough. Dough properties are part

of the selection criteria in the DBA program and thus new varieties are superior to an older variety, EGA Bellaroi which has relatively weak dough as shown by consistently lower MPT, GI and higher RBD (meaning less stable dough) than site means (**Figure 1** and **Supplementary Figure S2**). In contrast, Jandaroi consistently displays the highest GI (above mean at all 15 environments) and lower RBD (in this case lower or red coloured bars are better) with DBA Aurora close behind and less so, DBA Vittaroi (GI only). Undesirable low dough strength (GI) genotypes were 280115, 290564, 290491 (**Figure 1**).

Heritability for Non-Pasta Traits

Heritability estimates for non-pasta traits are represented in a boxplot (**Figure 2**). These give an indication of what the genetic variation is at an environment and are calculated for each environment hence a range in values is obtained. The narrower the range and higher the value, the stronger the G contribution to trait variation. Some parameters gave a narrow range in values with very high heritability such as b^*, WI and GI above 0.7 indicating stronger G contribution to trait variation. Milling traits (MY, SY. Semo) and a^* show more variability but still were mostly above 0.5. Other parameters showed a much wider range and interestingly WG showed a narrower range of variation (~0.3–0.9) compared with GP (~0.1–0.9). Selection is likely to be much more effective for a^*, b^*, WI, GI, milling and WG than other traits.
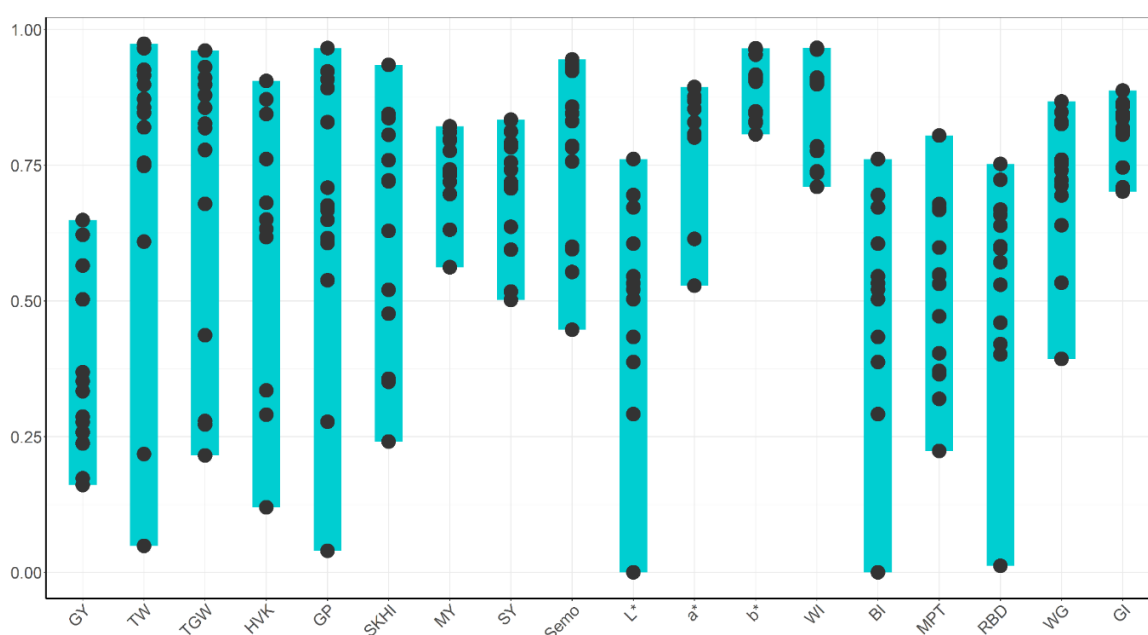

Figure 2. Heritabilities of the non-pasta traits across the environments. A single dot represents a heritability within an environment with the bars representing the complete range across environments.

Table 4. ANOVA Table of Wald statistics of fixed terms in each of the trait models for pasta traits. *P*-value significance of terms is represented by superscript stars (*** <0.001; ** <0.01; * <0.05). Bolded numerics in each column represent the highest order significant terms respecting marginality. Phenotypic trait minimums, maximums and averages are given at the bottom of the table.

Effect	DOF	DPL	DPa	DPb	DPWI	CPL	CPa	CPb	CPWI	CL	WABS	Firmness	SPH	SArea
Y	1	**226.7** ***	**164** ***	**22.1** ***	**125.6** ***	1.9	**36.1** ***	3.6	0.5	**55.6** ***	**632** ***	0.0005	**77.2** ***	**67.9** ***
L	3	**13.2** ***	1.7	6.2	12.8 **	13.2 **	1.9	1.7	4.2	18.9 ***	**54.5** ***	**13.7** **	1.3	0.4
G	5	8.2	28.5 ***	**71.2** ***	**26.8** ***	28.1 ***	38.4 ***	243.2 ***	200.8 ***	24.1 ***	**17.5** **	**42.6** ***	7.2	1.1
Y × L	1	1.4	0.02	0.9	1.9	7.9**	0.7	3.7	3.6	3.9*	0.07	0.7	0.3	0.003
Y × G	5	1.0	7.9	7.4	3.0	5.0	9.4	**20.1** **	**18.9** **	5.0	10.4	6.7	1.7	2.9
L × G	15	21.9	**32.6** **	14.6	18.3	113.5 ***	**29.9** *	**25.3** *	**34.3** **	**30.6** **	11.7	17.9	**27.9** *	**32.7** **
Y × L × G	5	9.1	6.7	7.4	8.9	**31.1** **	7.7	9.4	9.2	9.8	9.8	7.5	7.6	6.3
Min		69.9	−1.9	51.9	−84.5	76.9	−5.6	35.7	−72.0	5.4	158.7	660	22.7	10.8
Max		70.7	−1.0	56.7	−78.2	78.0	−4.9	41.7	−57.8	5.9	164.5	729	24.0	11.1
Mean		70.3	−1.4	54.6	−81.8	77.7	−5.3	39.0	−65.8	5.6	162.0	700	23.3	11.0

Effect of Genotype, Environment and Their Interaction on Pasta Quality Traits

The pasta traits shows a quite narrow range in BLUEs for key traits showing the good overall pasta quality for all the genotypes (**Table 4**). After cooking the pasta appears brighter but loses some yellow pigment (lower b^*). CL values were all excellent with a very narrow range while pasta firmness range is related to GP variation since these traits are correlated positively (**Supplementary Figure S4**). All genotypes had low and acceptable stickiness values. The Wald statistics for pasta traits shows only CPL having a significant Y × L × G interaction. Significant genotype effects were found for most color measures (DPL, CPL, DPb, CPb, DPWI, CPWI), CL, WABS and Firmness but either Y or L effects were also significant for these traits. While parameters with no significant G effect were stickiness and DPL.

Overall Performance and Stability for Non-Pasta and Pasta Traits

The overall performance (OP) and the stability (ST) of the genotypes is graphically displayed in **Figure 3** of non-pasta traits. For any given trait, the plots provide a useful summary of the relative overall performance across all the environments (further to the right on the x-axis equals higher OP) and stability (closer to the origin on the y-axis means more stable performance for the trait). For convenience, a numerical summary of the OP values of the genotypes across all the non-pasta traits is given in **Supplementary Table S3** with a summarised set of ST values in **Supplementary Table S1**.

Supplementary Table S3 highlights the best performing genotypes for each trait (bold numbers). DBA Lillaroi was well above average for TGW, SY and Semo that are linked to milling performance with average yield and above average GP and b^* while 290564 was well below average for TGW, SY and Semo having consistently poor milling performance. Varieties with above average score (large, positive deviation ≥ 0.4) in 4 or more traits are Jandaroi, DBA Vittaroi, DBA Aurora and DBA Lillaroi while none achieved OP > 0.4 for GY. If high dough strength is desired, for example to use as a parent in crossing, Jandaroi and DBA Aurora would be preferred; for high yield use DBA Aurora or Hyperno; for high milling potential use DBA Lillaroi. A compromise would be needed on deciding which genotypes to progress to a released variety. Decisions on what genotypes to progress depend on the market requirements in the target production zone.
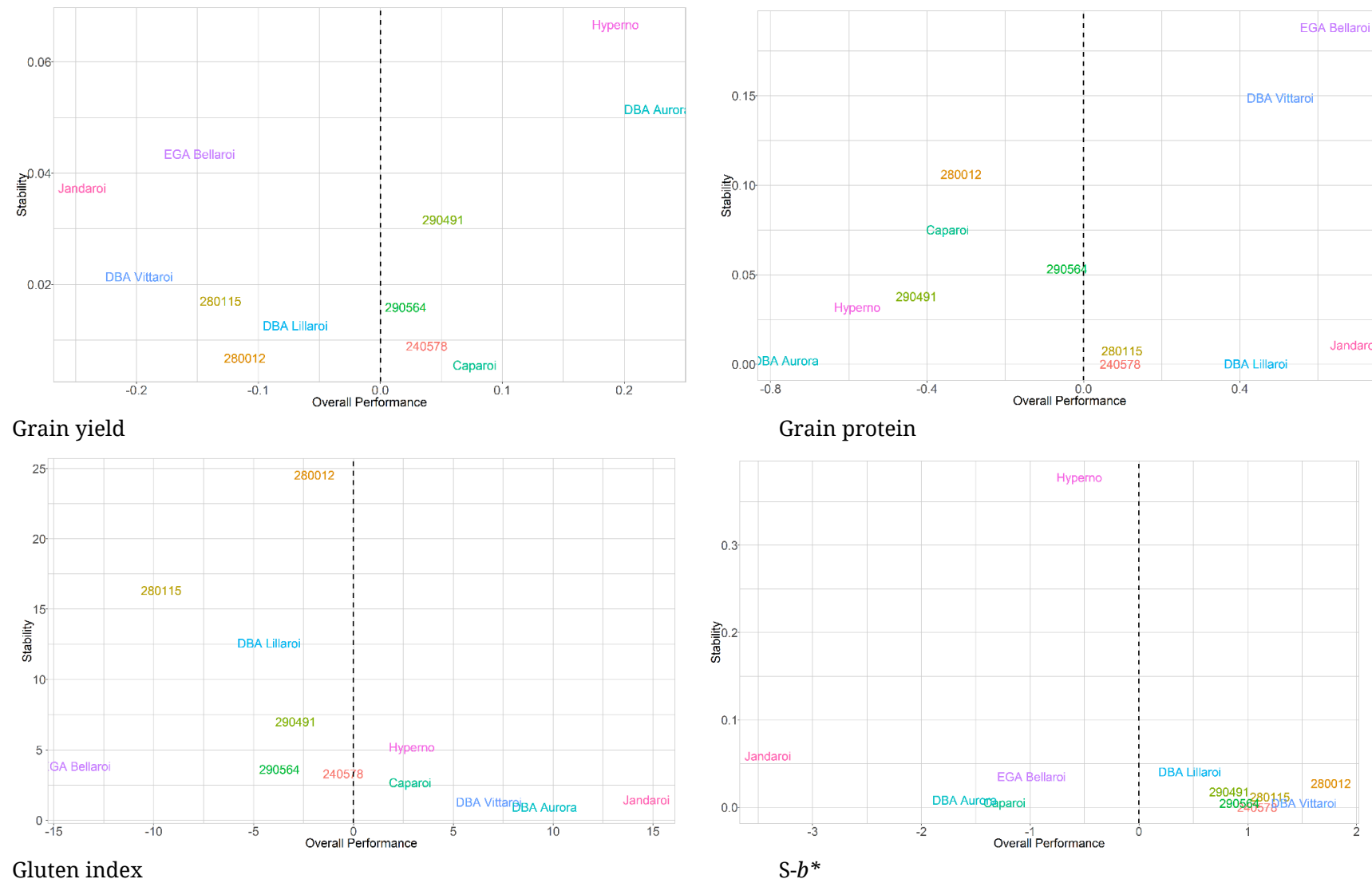
Grain yield

Grain protein

Gluten index

S-*b**

Figure 3. Plots of overall performance (OP) versus stability (ST) for a selected set of non-pasta traits. Varieties on the far right of the plot can be viewed as the best overall performers across environments and varieties approaching zero on the y-axis have increased stability across environments.
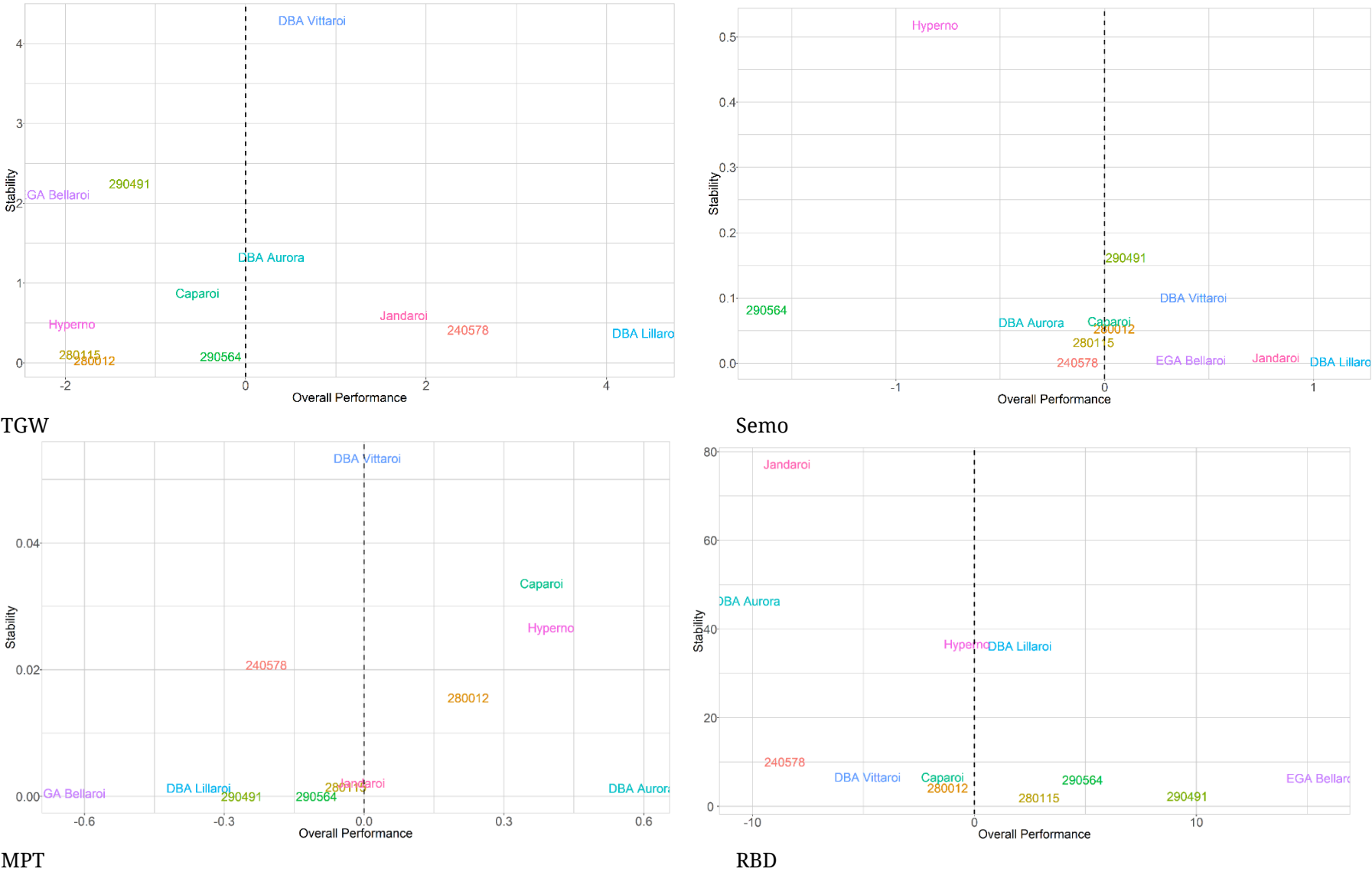
TGW

Semo

MPT

RBD
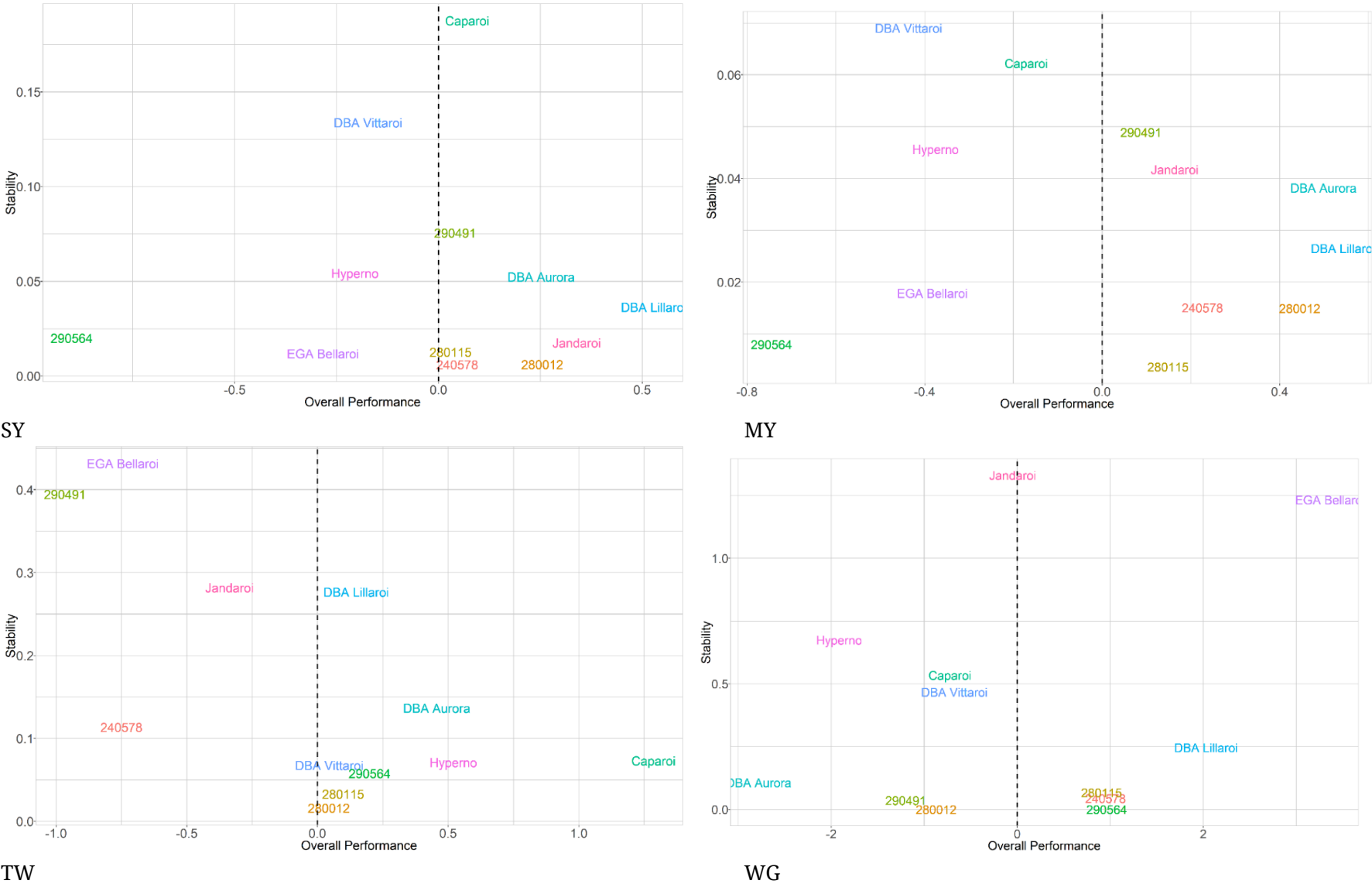
Figure 3. *Cont.*

SY

MY

TW

WG

Figure 3. *Cont.*

The graphical summaries of OP and ST given in **Figure 3** are a convenient way to illustrate genotype relative performance and would assist breeders in deciding on the best genotype. Clearly decisions are based on multiple parameters that need to be balanced. The most important criteria for a breeder and grower is grain yield and it is clear that both DBA Aurora and Hyperno are the standout varieties for yield across the environments (**Figure 3**). Comparing these two for quality shows DBA Aurora is superior to Hyperno for SY, Semo, TGW, Semo b^*, GI, MPT, RBD so would be preferred over Hyperno. However, the main issue for DBA Aurora for these 15 environments in NSW is the poor GP OP and tendency to higher screenings [29]. EGA Bellaroi was once a dominant variety grown in NSW is clearly now outclassed based on OP/stability for most parameters (GY, GP, GI, TGW, MPT, RBD).

Stability (ST) of genotypes across all environments can be quantified with the most stable having values approaching zero (**Supplementary Table S1**). Some genotypes were quite stable across all the traits with low values such as 240578, 280012 (except for GI) and 290564 while the most unstable with consistently high values for numerous traits are EGA Bellaroi, Hyperno, Jandaroi and DBA Vittaroi. The best genotypes for yield stability are Caparoi and 240578 and while OP for DBA Aurora and Hyperno were highest, their stability was worse than all other genotypes.

Relative performance for six durum wheats grown at six environments and evaluated for a range of pasta quality tests are presented as plots for key traits (**Supplementary Figure S3**). This summary can be viewed as a proxy for overall performance (OP) of the varieties. For good quality pasta desirable values for all the traits are high values for colour (DPL, CPL, DPb, CPb, DPWI, CPWI), cooked Firmness, FArea and WABS which means the blue boxes are desirable while for traits DPa, CPa, CSTAB, CL, SPH, SArea1 and SArea2 lower values red boxes are desirable. Pasta colour is closely related to semolina colour, indeed there was a significant correlation between S-b^* and DP-b^* and CP-b^* (**Supplementary Figure S4**) but only poor correlations between the corresponding L^* and a^* values. There is also a high correlation between DPb* and CPb* (r = 0.89, p < 0.001) which shows that much of the pigment is retained after cooking. Varieties with below site mean S-b^*, like Jandaroi, Caparoi and DBA Aurora also had lower DPb* and CPb* performance. Pasta appearance is affected by not just b^* but also L^* and a^* so that although EGA Bellaroi achieved above average DPb*, the DPa* was redder making the pasta appear duller to the naked eye. Pasta texture

was assessed by cooked firmness, overcooking tolerance and cooked stickiness. DBA Lillaroi, EGA Bellaroi, and Jandaroi tended to higher firmness while other genotypes showed more variability. Overcooking tolerance relates to firmness after 10 min overcooking, with a small reduction being desirable. Hence orange bars are preferred. Genotypes with higher firmness (and GP) like EGA Bellaroi and Jandaroi had higher colour stability (CSTAB) and DBA Aurora with lower firmness also had better CSTAB showing the traits were not correlated. However, there was very little variation in CSTAB across genotypes with no significant differences in means. There were no clear consistent trend in stickiness across the genotypes showing variable responses across environments. But predicted values show all genotypes had acceptable stickiness. DBA Aurora consistently achieved higher levels than the overall mean across environments while 290491 tended to lower values.

The OP values for the pasta traits are found in **Table 5** and ST in **Supplementary Table S2**. The best performing genotypes for each trait are bolded. Values with a higher DPL, DPb, CPL, CPb, WABS, Firmness, FArea and traits with lower values for DPa, CPa, DPWI, CPWI, CSTAB, CL, SPH, SArea1 and SArea2 are desirable. The genotype with the best colour parameters was 290491 followed by DBA Aurora, which also produced the least firmness and highest SPH. For ST values close to zero represent the greatest stability across the test environments. Caparoi showed the best ST for most of the colour traits except CSTAB while Jandaroi had the least ST. EGA Bellaroi had the best ST for CL and stickiness while Jandaroi was best for firmness ST.

Trait Correlations for Non-Pasta and Pasta Traits

The BLUEs values for all traits were subject to pairwise correlation analysis and results are shown as a pictogram (**Supplementary Figure S4**). A strong positive correlation ($r > 0.6$) was obtained between GP and WG and negative correlations between GI and RBD/WG; MPT and RBD/WG/HVK; $L*$ and GP; WG and SY although any $r > 0.2$ was significant, $p < 0.01$ but in practice do not explain much of the variation.

Table 5. For all pasta traits, the relative performance of BLUEs averaged across environments. This summary can be viewed as a proxy for overall performance (OP) of the varieties. Bold values represent the best overall performing genotype for each trait analysis.

Genotype	DPL	DPa	DPb	DPWI	CPL	CPa	CPb	CPWI	CSTAB	CL	WABS	Firmness	FArea	SPH	SArea1
EGA Bellaroi	0.094	0.537	1.195	−1.676	0.093	0.253	0.803	−1.776	0.350	−0.014	−1.701	**29.046**	**0.046**	−0.219	0.009
Caparoi	−0.145	0.004	−0.935	1.523	**0.315**	−0.025	−0.578	0.811	−0.137	0.012	1.678	−9.875	−0.027	**−0.425**	**−0.014**
DBA Aurora	**0.225**	**−0.507**	−0.454	0.052	0.179	**−0.308**	−0.461	0.723	−0.033	0.348	**2.164**	−40.091	−0.063	0.668	−0.006
DBA Lillaroi	−0.288	−0.124	0.782	−0.437	0.021	−0.092	0.905	−1.542	−0.035	−0.090	−0.959	16.484	0.034	0.158	−0.001
290491	0.063	0.076	**2.106**	**−2.886**	0.177	−0.267	**2.636**	**−6.179**	**−0.290**	**−0.198**	−0.950	−6.697	−0.007	−0.240	0.013
Jandaroi	0.052	0.015	−2.695	3.425	−0.785	0.440	−3.305	7.963	0.145	−0.058	−0.232	11.133	0.017	0.058	−0.001

DISCUSSION

To date there have been no reports of a G × E study for durum wheat quality traits in the Australian dryland production environment. We have used modern statistical approaches to analyse data obtained from a G × E study consisting of 15 environments. While the genotypes tested may become less relevant as breeders release new varieties, the approach used in this study will provide durum wheat breeders with vital information on trait heritability as well as tools to measure genotype performance and stability across environments.

Effect of Genotype, Environment and Their Interaction, Stability and Overall Performance on Grain Yield and Non-Pasta Quality Traits

Phenotype is a product of the genotype, environmental influences (rainfall, temperature, abiotic and biotic factors, soil conditions and management practices) and the interaction of these two factors (G × E). In breeding the aim is to develop better genotypes in terms of improved trait(s) while also ensuring good stability of performance across multiple environmental conditions. G × E studies are important in evaluating cultivar adaptation, selecting parents for crossing, and developing improved genotypes. If the ranking of genotypes differs between environments, identification of superior breeding genotypes is hampered because values are affected more by environmental variation than genetic differences. Such knowledge has the potential to reduce the number of test environments required to provide assurance a genotype is performing well across the range of environments where durum wheat can be grown in NSW relative to control varieties with long term demonstrated performance. To provide detailed interpretation of the G × E interaction effects for each of the traits we have used a combination of two highly structured multi-environment linear mixed models (ME-LMM). For pasta and non-pasta traits, we initially modelled the G × E interaction as a fixed component in the ME-LMM to provide accurate hypothesis testing as well as to provide a detailed summary of the G × E effects through relative variety performance between and within environments. For non-pasta traits a second ME-LMM was then fitted that used a Factor Analytic model for the G × E interaction effects [18,19], allowing a newly developed FAST approach [18] for accurately determining variety stability across environments.

In our G × E study, we measured key traits used in the Durum Breeding Australia (DBA) program on 12 genotypes representing commercial and advanced breeding genotypes. All the phenotypic responses were recorded (**Supplementary Figure S1**) which shows the variability obtained in this study across the 15 environments. Some traits show little variation (narrow box plots) like BI, MPT and *L** while others much more. Environmental conditions like rainfall, temperature during grain filling and at maturity together with the genotype interaction underly the phenotypic variation observed. The ANOVA tables showed that almost all non-pasta traits had significant G × E variation (**Table 3**). Also, many traits were influenced more by G than Y × L (TW, TGW, GP, *a**, *b**, WI, WG, GI). This is reflected in the heritability estimates with many showing a wide range although GI, WI, *b**, *a** Semo, SY, MY have a narrower range with values 0.45–0.95 (**Figure 2**). While TW, TGW, GP and WG all showed a strong genotype effect (**Table 3**), their heritabilities were still highly variable (**Figure 2**) indicating significant environmental effects in some environments whilst genetic effects dominate these traits in an overall sense. This shows a strong potential for genetic gain through breeding and selection for these traits (GI, WI, *b**, *a** Semo, SY, MY). Selection for these traits in early generations could be effective provided test methods suitable for small grain samples with large sample throughput are available. Other studies in durum wheat show more influence of environment on GP, WG, yellowness, HVK, TW and TGW but agree with our finding of high E variation for GY and HVK [11,30]. For GP heritability, estimates have been reported as low to moderate [31,32] while others [30,33,34] obtained values ~75%, (no range provided). Heritability and G × E effects on durum yield and quality traits will vary between studies due to use of different genetic material, environments and methods of statistical analysis used [35]. What matters to the breeders is the use of environments that are representative of the durum production zones. Results from this study indicate that genetic gains in GI, WI, *b**, *a** Semo, SY, MY will be possible.

In a study of 23 durum wheats grown in Ethiopia in 2010 it was noted that TGW was a good predictor of GY with TGW having a high heritability of 81% [36]. However, our data shows very low correlation (**Supplementary Figure S4**) between these traits although TGW showed high G contribution and variable but reasonable heritability (**Table 3** and **Figure 2**). GY is strongly related to grain number [37,38]. Typically yield and grain protein are negatively correlated

due to the dilution effect on grain being larger with higher yield (grain with more carbohydrate), so proportionately has less protein [39]. The correlation between GY and GP was $r = -0.43$, $p < 0.05$ following the expected trend. WG and GP are highly correlated ($r = 0.93$, $p < 0.001$ (**Supplementary Figure S4**) because ~80–85% of grain protein in wheat is gluten [40], hence WG was also negatively correlated with GY ($r = -0.45$). Generally, genotypes with high OP for GY were lower for GP (**Figure 3**, **Supplementary Table S3**) and those about the origin in **Figure 3** (performance vs stability plots) were as expected, between these extremes. Given the importance in achieving GP for grading purposes (13+%, 11%mb in Australia), clearly a compromise on GY in favour of GP is needed.

Grain vitreousness is another important commercial trading measure with high levels of vitreosity >80% leading to good semolina yield and pasta of good appearance [41]. Overall HVK mean was >80% except at 2015-Tamworth and 2015-Narrabri possibly due to weather conditions such as high rainfall during grain filling, although 2015-Narrabri also had lowest GP and both traits are negatively correlated ($r = -0.31$, $p < 0.01$). Rainfall events can lead to reduced HVK [42]. There were high levels of rainfall in November at these environments (**Supplementary Figure S5**) although similar rainfall was observed in other environments so other factors are important. GP was lowest at 2015-Narrabri which would reduce HVK at this site. Vitreous kernels are characterized by a natural translucent colouring or "glassy" appearance. Vitreosity is linked to protein content [43] which affects grain structure with higher protein creating a more compact grain structure of starch granules surrounded by a gluten matrix with fewer air spaces resulting in a vitreous kernel appearance. However, HVK is highly affected by environmental conditions during grain development (rainfall) which explains the high Wald values for Y, L and Y × L for HVK. Other studies confirm the greater impact of environment than genotype on this grain parameter [5,11,30,44]. DBA Lillaroi showed a more frequent lower HVK than the overall mean across 9/15 environments (**Supplementary Figure S2**), indicating that should rainfall occur near harvest, this variety could be more susceptible to lower HVK and consequent downgrading and price penalty. However, since the release of DBA Lillaroi grower experience has been positive with no instances of issues with low HVK reported to date.

Grain density and weight affects important grain measures, TW and TGW which are related to semolina yield with TW thought to be a better indicator in durum [45]. DBA Lillaroi gave consistently the highest semolina yield measured as SY or Semo and this is more likely related to the very large grain size this genotype produced (highest and most consistent TGW across environments) even though TW data does not suggest a good milling potential. It seems under Australian conditions, TGW is a better predictor of milling yield than TW as the high TGW performers, DBA Lillaroi and Jandaroi were also the highest for milling yield (SY vs TGW, r = 0.46 compared to SY vs TW, r = 0.28, **Supplementary Figure S4**). In contrast, 290564 was consistently lower for SY and Semo which were not obvious from the good TW and TGW (**Figure 3**). This suggests that other factors are important in determining milling potential [1] and that test milling is critical for breeders to improve milling yield. DBA Lillaroi was noted above as having good GP and acceptable GY but clearly has outstanding milling performance and these factors were instrumental in its release. DBA Lillaroi has enjoyed very high adoption upon its release representing ~41% of durum silo receivals in NSW [46].

Colour is an important measure for the appearance and consumer acceptability of pasta which should be bright, yellow and not red with the three key measures being L^*, a^* and b^* used to assess this. Semolina yellowness is commonly used in breeding programs to select for pasta yellowness without the expensive and time consuming work to prepare pasta. Yellowness of semolina (S-b^*) is a good predictor of pasta b^* but not always [47]. Our study using a reduced set of samples for pasta making showed a high correlation between S-b^* and both DPb* and CPb* (r = 0.90 and 0.95, p < 0.001, respectively). Since b^* has high heritability (**Figure 2**), breeders have been successful in selecting for increased semolina yellowness world-wide [48]. Breeding for increased semolina b^* has been a key quality target for DBA for over 25 years and this is evident in the more recently developed genotypes (DBA Lillaroi, 240578, 280012, 280113, DBA Vittaroi) compared with the older varieties like EGA Bellaroi, Jandaroi, Caparoi, (released in 2003, 2007, 2008, respectively) as shown by their higher OP for semo b^* (**Figure 3**). Interestingly, all genotypes except Hyperno showed good ST values for S-b^*, showing that in Hyperno colour expression is partly dependent more on E than for the other genotypes in these environments.

Dough strength is thought to be important to pasta quality especially when protein is below industry recommended levels of 12–13% grain protein content [25]. Generally, durum wheat high in protein and with strong gluten ensures pasta is firm, retains its *al dente* texture with overcooking and is non-sticky with excellent culinary quality [49]. Dough properties were assessed by mixograph (peak mixing time and resistance breakdown or dough stability, with longer MPT and lower RBD associated with stronger dough) and gluten index (where values >70 are considered strong and >90 very strong) [25,50]. The GI method clearly differentiated genotypes into very strong (>90) to moderate strength (70–80). MPT seemed less discriminating with a narrower range in values but still with significant genotype differences. GI was significantly positively correlated with MPT (r = 0.50, p < 0.001) and negatively with RBD (r = −0.53) (**Supplementary Figure S4**). Heritability estimates shows that GI has a higher heritability than MPT and RBD (**Figure 2**) with higher genotypic effects (**Table 3**). Selection for increasing gluten strength is then best done using the GI measurement. The OP and ST for GI was highest for Jandaroi and DBA Aurora. The lowest OP was for EGA Bellaroi but ST was worse for 280012 (**Figure 3**, **Supplementary Table S1**). Since its release (2007), Jandaroi has consistently shown high GI values and EGA Bellaroi low values in the DBA program (unpublished data) consistent with this study. Interestingly, there was a small negative correlation between GP and GI. Late sown durum wheat is associated with increased protein content and GI [51,52] due to higher glutenin to gliadin ratio. However, higher grain protein may result in higher gliadin to glutenin ratio, which can reduce GI [53,54].

Effect of Genotype, Environment and Their Interaction, Stability and Overall Performance on Pasta Quality Traits

Many studies have not included end products in their G × E studies, considered by many as the ultimate way to assess wheat quality. Key pasta traits are a bright yellow colour with minimal colour loss after cooking. Cooked pasta should have a firm texture and retain its *al dente* even with overcooking and with low stickiness, minimal amounts of solids lost into the cooking water (low cooking loss) with a 2–3 fold increase in weight from water uptake (water absorption) to ensure a good mouthfeel [49].

Colour selection would be based on uncooked, dry pasta (DPL, DPa, DPb) and genotypes with the best OP for DPb were 290491, DBA Lillaroi and EGA Bellaroi (**Supplementary**

Figure S3). Of these, only the first two had above average OP for S-b^* (**Figure 3**) reflecting the moderate correlation between these measures, as described previously. Genotypes showing poor OP for S-b^* were Jandaroi, DBA Aurora, Caparoi and EGA Bellaroi (**Figure 3**) and these also performed below average for DPb except EGA Bellaroi. CPb followed a similar trend to DPb reflecting similar loss of yellow pigment during the cooking process (colour stability) between genotypes with EGA Bellaroi being slightly better (**Supplementary Figure S3**). Cooked pasta firmness is an important trait and generally firmer is better although this is influenced by GP. Firmness was correlated with grain protein (r = 0.587, p < 0.001). Genotypes with higher OP for GP (EGA Bellaroi, Jandaroi and DBA Lillaroi) tended to also have higher firmness (**Supplementary Figure S3**) showing that selection for this trait depends heavily on GP achieved and needs to be considered. Tolerance to overcooking (10 min after optimum) is important since this is commonly done by consumers so retaining the *al dente* mouthfeel is important. Absolute values show all genotypes were good although those with higher firmness tended to have better tolerance to overcooking. There were minimal differences in the mean BLUEs for stickiness peak height where lower values are desirable ranging from 22.7 to 24.0 g (data not shown). Stickiness arises during the cooking process as the pasta releases exudates from the starch granule gelatinization and amylose is thought to be mostly responsible [55]. These difference are not considered significant in practice, despite variation across environments (**Supplementary Figure S3**). The same was the case for CL where again, a narrow range in mean BLUEs of 5.4–5.9% was observed. The solids lost into the cooking water should be minimal with good pasta having up to 7–8% considered acceptable [56]. Overall, the pasta data shows all genotypes produced an acceptable product but taking into account all measured traits, the better genotypes were DBA Lillaroi and 290491. It should be noted that all these trait measures do not assess the sensory acceptability which covers visual appearance, taste, aroma and texture. Subjective assessment shows that EGA Bellaroi produces a duller pasta than DBA Lillaroi and DBA Aurora. The bright yellow pasta colour of DBA Lillaroi has been appreciated by the Australian pasta makers and it has met their requirements for a brighter yellow colour and opened new market opportunities [45].

CONCLUSION

This G × E study presents a comprehensive analysis of durum varieties and breeders advanced genotypes for grain yield, grain, semolina, dough and pasta making quality in the Australian dryland environment. A summary of the overall performance and stability of the genotypes is presented to assist identification of the best genotypes for each trait. A breeder needs to ideally combine several commercially important traits into one genotype and the emphasis given depends on the intended use. In the DBA program emphasis is on high yield and grain quality meeting the requirements of the value chain users (millers, pasta makers, marketers and consumers). While nearly all grain and semolina traits showed significant G × E only one pasta trait did (CPL). For non-pasta traits, good heritability was observed for traits such as milling yields, semolina $b*$, semolina whiteness and gluten index. Selection for these traits will enable genetic improvements. The OP vs stability plots allow at a glance the easy identification of superior genotypes having both a high OP and ST for as many traits as possible but usually a compromise is needed. However, this study ignored other trait data that is important for variety release that was beyond the scope of the study e.g., screenings, disease ratings, lodging, maturity etc. The limitations of our study were that there was no replication of the milling in individual trials and the small number of samples evaluated for pasta making quality, both due to resource limitations. However, this is the first detailed G × E study conducted on durum wheat in Australia. Overall, multi-environment data allows the heritability of traits to be determined and genotypes overall performance and stability allowing more quantitative comparisons. This will ensure better decision making about genotypes to develop and will achieve better productivity for the durum industry. We would recommend for industry the following genotypes based on this study that combine high performance and stability for both yield and many technological quality traits suited to the dryland durum growing regions of northern NSW, Australia: DBA Lillaroi and breeding line 240578. Furthermore, DBA Lillaroi possesses a bright semolina and pasta colour which is attractive to the durum milling and pasta making industry.

SUPPLEMENTARY MATERIALS

The following supplementary materials are available online at https://doi.org/10.20900/cbgg20200018:

Supplementary Figure S1: Boxplot of trait BLUEs showing variation for non-pasta traits across environments. Boxplots show the median and interquartile range of trial data.

Supplementary Figure S2: Selected plots showing the best linear unbiased estimator (BLUEs) values expressed as deviations around the environment (site x year) mean of 12 durum wheats across 15 environments for non-pasta traits. For any given trait, muted blue bars indicate better than average performance of a variety and muted red bars indicates worse than average performance except for RBD which is the reverse.

Supplementary Figure S3: Plots showing the BLUEs values expressed as deviations around the site mean of 6 durum wheats across 6 environments for selected pasta quality traits.

Supplementary Figure S4: Pairwise correlation plot for non-pasta traits between BLUEs of the traits.

Supplementary Figure S5: Rainfall and minimum/maximum temperature data from all the environments used in the study.

Supplementary Table S1: Numerical stability of genotypes (ST) across all environments for a selected set of non-pasta traits. For each trait, values were extracted directly from the stability analysis and scaled by the standard deviation across the genotypes for more suitable comparison. Genotypes with values approaching zero exhibit greater stability across environments. Bold values represent the most stable genotype for each trait analysis.

Supplementary Table S2: For all pasta traits, a summary of mean squared deviation of BLUEs from their relative average performance across environments. This summary can be viewed as a proxy for variety stability (ST).

Supplementary Table S3: Numerical overall performance of genotypes (OP) across all environments for a selected set of non-pasta traits. For each trait, OP values were extracted directly from the stability analysis. Bold values represent the best overall performing genotype for each trait analysis.

DATA AVAILABILITY STATEMENT

The dataset of the study is available from the authors upon reasonable request.

AUTHOR CONTRIBUTIONS

MS conceived the study, managed the project and the laboratory testing, wrote the original article; GK managed the field trials; JT performed all the statistical analysis. All authors reviewed and approved the final version.

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