Strengths and Weaknesses of National Variety Trial Data for Multi-Environment Analysis: A Case Study on Grain Yield and Protein Content

Vahid Rahimi Eichi [1,2], **Mamoru Okamoto** [1,2], **Trevor Garnett** [1,2,3], **Paul Eckermann** [1,2], **Benoit Darrier** [1], **Matteo Riboni** [1] **and Peter Langridge** [1,4,*]

1 School of Agriculture Food and Wine, University of Adelaide, Glen Osmond 5064, Australia;
vahid.rahimi-eichi@adelaide.edu.au (V.R.E.); mamoru.okamoto@adelaide.edu.au (M.O.);
trevor.garnett@grdc.com.au (T.G.); paul.eckermann@adelaide.edu.au (P.E.);
benoit.darrier@adelaide.edu.au (B.D.); matteoriboni@hotmail.it (M.R.)

2 ARC Industrial Transformation Research Hub for Wheat in a Hot and Dry Climate, Waite Research Institute, University of Adelaide, Glen Osmond 5064, Australia

3 Australian Plant Phenomics Facility, The University of Adelaide, PMB1, Glen Osmond, South Australia 5064, Australia

4 Wheat Initiative, Julius-Kühn-Institute, 14195 Berlin, Germany

* Correspondence: peter.langridge@adelaide.edu.au; Tel.: +61-8-83137368

Abstract: Multi-environment trial studies provide an opportunity for the detailed analysis of complex traits. However, conducting trials across a large number of regions can be costly and labor intensive. The Australian National Variety Trials (NVT) provide grain yield and protein content (GPC) data of over 200 wheat varieties in many and varied environments across the Australian wheat-belt and is representative of similar trials conducted in other countries. Through our analysis of the NVT dataset, we highlight the advantages and limitations in using these data to explore the relationship between grain yield and GPC in the low yielding environments of Australia. Eight environment types (ETs), categorized in a previous study based on the time and intensity of drought stress, were used to analyze the impact of drought on the relationship between grain yield and protein content. The study illustrates the value of comprehensive multi-environment analysis to explore the complex relationship between yield and GPC, and to identify the most appropriate environments to select for a favorable relationship. However, the NVT trial design does not follow the rigor associated with a normal genotype × environment study and this limits the accuracy of the interpretation.

Keywords: National Variety Trials; grain protein content; multi-environment; grain yield; high and low protein wheat; environment type; grain protein deviation

1. Introduction

Wheat is a major source of protein in the human diet and, along with rice, the most important crop product for carbohydrate [1,2]. Meeting the food demands of an estimated 9 billion people in 2050 [3,4] requires about 70% increased production over the next 30 years [5,6]. Through advances in agronomic practices and breeding, wheat yields have increased spectacularly over the past few decades [2,4,5]. Many of the major traits, such as yield and GPC, targeted for improvement in breeding programs and of prime importance to farmers, are under complex genetic control and strongly influenced by the production environment. For both wheat breeders and farmers, the relationship between yield and GPC is important. Consequently, many countries run extensive varietal trials to provide information for farmers on performance of varieties under their likely production conditions and for

breeders to assess the advances they have achieved through breeding and selection. The variety trials also provide a potentially valuable resource for researchers to understand the basis of genotype × environment relationships.

　　Concurrently, grain protein content (GPC), a key attribute for end use quality with high nutritional value and an important marketing factor for wheat, will also have to be maintained [7–9]. For example, in Australia, GPC is used in determining the value of the wheat crop to farmers [10]. However, there is an unfavorable relationship between grain yield and GPC with increased yield frequently associated with a decline in GPC and this presents a significant challenge to breeders [9,11,12]. A study on winter wheat in Germany showed that good progress in raising grain yield was associated with a considerable loss in GPC over the last 32 years [9]. Consequently, wheat germplasm is often divided into high yielding or high quality varieties, as defined by high GPC% [13]. To simultaneously improve both grain yield and GPC, grain protein deviation (GPD) has been suggested as a useful selection target [14,15]. Grain protein deviation measures the grain yield/GPC trade-off by compensating for GPC dilution when selecting for higher yield potential [16]. Grain yield and GPC in wheat have low heritability since they are highly sensitive to environmental variations and under complex genetic control [9,17]. Therefore, despite evidence for the genetic origin of the negative relationship between grain yield and GPC in wheat [9,18], its expression can be highly influenced by the environment [11,19]. A previous study using six Southern and Western Australian sites showed that the inverse relationship between grain yield and GPC varied across different regions [20]. Therefore, multi-environment trial studies can provide an opportunity for the simultaneous selection of both grain yield and GPC [11,19,20]. However, conducting trials across a large number of regions can be costly and labor intensive. In this context, Australian National Variety Trials (NVT) funded by Australian Grains Research and Development Corporation (GRDC) provide a useful source of multi-environment data. The NVT system provides information to growers through the NVT website (https://www.nvtonline.com.au/) to assist with decisions about suitable varieties for particular regions in Australia. NVT data has been used in previous studies to examine the relationship between grain yield and GPC in some hard wheats [21,22]. The NVT system in Australia is similar to varietal trials conducted in many other countries and regions, such as the UK (https://ahdb.org.uk), Germany (https://www.bundessortenamt.de), and Kentucky, USA (http://www.uky.edu/Ag/wheatvarietytest/).

　　NVT are designed to provide unbiased information on the performance of varieties across different Australian regions [23]. Deploying small plots in NVT trials minimizes the large-scale field variation effects. Accordingly, a combination of the small plot trials with modern statistical methods have provided an accurate way to evaluate the performance of varieties [24]. The rationale for all NVT trials is to achieve the best performance of varieties within the constraints of water-limited yield potential under management regimes suited to the trial region. In NVT trials, optimal fertilizer rates are usually applied to prevent the nutrition limitation [24] along with other appropriate agronomic practices. Environmental categorization of NVT sites adds the option of characterization of genotype and environment interactions [25] and comparison of biotic and abiotic stresses [26,27]. The majority of the Australian wheat-belt area suffers from drought conditions [28]. Drought stress may reduce the production of wheat up to 50% depending on its severity and duration [29]. It has been shown that grain yield reductions due to drought can increase the GPC in wheat [30–32]. Based on the time and intensity of drought stress, Chenu and Deihimfard [28] categorized different drought patterns across the Australian wheat-belt into four major environment types (ETs). Accordingly, ET1 represents a stress-free or short-term water deficit. ET2 shows a mild water shortage mainly during grain filling that terminates by maturity. In ET3 water stress is severe at the vegetative stage but is usually over by mid-grain filling. In ET4, water deficit begins from the early stage onwards, and becomes severe during grain-set and grain filling [28]. All of the four ETs can be seen across most the Australian wheat-belt. However, the frequency of occurrence for each ET varies between different regions. In this study, 11 years of NVT dataset were categorized based on their most frequent ETs. Through the use of the NVT data, we have sought to highlight the advantages and limitations of using a large multi-environment

datasets. We present a case study of applying the NVT data to explore the negative grain yield–GPC relationship across diverse environments. If there is variation associated with the environmental conditions, and under what conditions should breeders screen high yielding germplasms for high GPC? Our previous study in a controlled environment indicated a stronger inverse relationship between grain yield and GPC under low nitrogen (N) treatment in selected wheat varieties [33]. Since N deficiency can be associated with water availability [34,35], it is worthwhile to search the effect of ETs on the grain yield–GPC relationship. The goals of this study were: (1) assess the potential of using NVT data for large-scale multi-environment analysis and (2) to see if GPC is a stable trait across different environments. The lessons learned here, should be applicable to other varietal evaluation datasets and could be used to address some of the limitations in the current design of varietal trials.

2. Materials and Methods

2.1. General NVT Protocols

Trials analyzed in this study were organized and managed under the GRDC NVT program between 2008 and 2018 for 215 wheat (*Triticum aestivum* L.) varieties in 206 sites across the Australian wheat-belt and Tasmania (Supplementary Figure S1). At some sites, a year could include up to three seasons described as early, main or late seasons. However, only 2% of the entire data set are obtained from long seasons. There is 79% and 18% of the data from main and early seasons, respectively. Based on the popularity of varieties with growers in each region, newly released varieties could remain for up to 5 years in an NVT system. Subsequently, varieties that represent less than 3% of the annual yield are withdrawn from the NVT list. NVT breeding materials comprise varieties prior to their commercial release and released varieties. Commercial varieties that are widely grown in a region, e.g., Mace in South and Western Australia, are used as benchmark varieties [23].

Selection and management of NVT sites are determined by standard, outcome-based protocols. However, additional fertilizer and pesticides are applied when required [23]. In this context, fertilizer rates applied for NVT trials are often higher than the commercial rates in the region [24]. All NVT trials are designed with three replicates but only grain yield is obtained from the individual plot. Due to its high cost, GPC is assessed by using composite samples. In this method, equal weight grain samples collected from each replicate are physically mixed to form a homogenous composite unit. GPC measurements are made on the subsample, with an appropriate size, of each of these composite units [36].

2.2. Multi-Environmental Analysis and Graph Depiction

Chenu, Deihimfard [28] characterized 60 sites by simulated water-stress index obtained from their climate and the typical soil of their region (Supplementary Table S1). Simulated water-stress index corresponds to the ratio of soil water supply to crop water demand, and reflects how the crops experience the stress [28,37,38]. In the mentioned study, they performed a set of simulations for a medium maturing variety, Hartog, based on 123 years of historical climate data obtained from 22 regions across the Australian wheat-belt (Supplementary Figures S2 and S3). The 22 regions represent the major production areas in the Australian wheat cropping system. Data shown in Supplementary Table S1 are mostly obtained from Ababaei and Chenu [39] simulations on Janz variety.

Among the 206 NVT sites examined in this research, 46 sites were identical with the locations in Chenu, Deihimfard [28] study. The location of other NVT sites were individually checked to determine their regions and ETs. Except for Tasmania, all mainland NVT sites were located in the recognized 22 regions [39].

Categorizing NVT data based on ETs requires additional information, such as cumulative rainfall and soil type, for all 206 individual sites over 11 years. Furthermore, some sites could cover multiple seasons in one year due to differences in sowing date. Consequently, in this study, individual sites were categorized based on the most frequent ETs in their regions (Supplementary Information). This can

be an efficient and quick method to provide an overview of the long-term drought stress conditions across the Australian wheat-belt. NVT datasets are available for a range of consecutive years in similar locations. Therefore, the average long-term results for yield and GPC could be more influenced by the dominant ETs compared to other ETs in each region. Accordingly, NVT sites, except for Tasmanian sites, were categorized based on their dominant ETs in each region. In this research, the number of sites for each of 22 regions ranged from 4 to 32, while they were between 1 and 8 in Chenu, Deihimfard [28] study. The map of frequencies of each ET across the Australian wheat-belt [28] were used to categorize different regions (Supplementary Figure S4).

Grain yield and GPC between different ETs were compared based on their average and median values. *p*-Values and statistical tests were not conducted due to potential limitations with the analysis of NVT dataset. These limitations are explained in the discussion section. Correlations between grain yield and GPC were assessed using Pearson's correlation coefficient. The slope of grain yield–GPC relationship was measured for each ET by averaging the grain yield–GPC slope from individual sites in each year. GPD was calculated for individual sites in each year as the residual from the regression line of grain yield–GPC relationship [14]. All the analyses and the graphics were performed using R v3.5.1 [40].

3. Results

Based on the map of the frequencies of each ET across the Australian wheat-belt [28], there were two dominant ETs in some areas (Supplementary Figure S4). Such regions were categorized as separate ETs since they lie in between of the two ETs. For instance, regions with dominant ET1 and ET2 were named with ET1/2. Accordingly, there were eight ETs for NVT data in this study: ET0, ET1, ET1/2, ET2, ET2/3, ET3, ET3/4, and ET4. In this context, ET1/2, ET2, and ET2/3 covered respectively, 20%, 26%, and 24% of the entire NVT data set (Table 1).

Chenu and Deihimfard [28] did not include Tasmania in their study. However, there were NVT sites near Launceston where the average rainfall was ~600 mm per year [41]. Accordingly, the datasets of these sites located in the high rainfall zones (HRZ) of Tasmania were categorized as ET0 in this study. Average grain yield for the different environments ranged from 9.2 t/ha for ET0 down to 1.6 t/ha for ET4 (Figure 1A).

Table 1. General information about National Variety Trials (NVT) dataset. The average number of varieties per site, number of years, total number of sites during consecutive years, and total number of data in individual environment types (ETs).

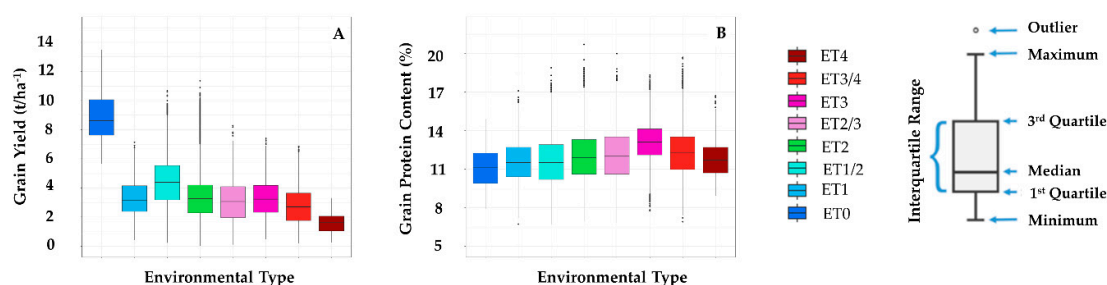| Environment Type	ET0	ET1	ET1/2	ET2	ET2/3	ET3	ET3/4	ET4
Average no. of varieties per site	20	32	41	43	40	43	38	28
No. of years	7	11	11	11	11	11	11	11
No. of sites × year	7	132	254	321	317	85	193	29
Total no. of variety × site × year × season	140	4263	10,347	13,766	12,597	3666	7237	805

Figure 1. Grain yield (**A**) and grain protein content (**B**) of all 215 varieties in the NVT dataset. Total numbers of variety × site × year × season were 52,822 collected from 206 sites. Name of varieties is listed in Supplementary Data S1. Boxes refer to the 25th (first quartile), 50th (median), and 75th (third quartile) percentile of data.

The decline in in-season rainfall from ET0 to ET4 corresponds to the declining average yield (Figure 1A and Supplementary Table S1) with ET1 being an exception, most probably associated with the low soil fertility and short season in the ET1 environments of Western Australia [42–44]. After the HRZ of Tasmania (ET0), areas with dominant ET1/2 produced the highest grain yield compared to other ETs (Figure 1A). ET1/2 dominant regions are located in the HRZ of South Australia, Victoria and New South Wales, whereas ET1 areas are in the HRZ of Western Australia. ET1/2 and ET1 regions produced 4.4 and 3.3 t/ha, respectively. There were data available for most ETs during all 11 years except for ET0 where only 7 years of NVT trials were available.

GPC average appeared to increase from ET0 to ET3, and then decline towards ET4 (Figure 1B). The GPC mean of ET0, ET3 and ET4 were 11.1%, 13.1%, and 11.8%, respectively. This variation may not be entirely due to environmental effects since the variety lists did vary between different ETs. There were inverse relationships between grain yield and GPC when the entire NVT dataset was plotted (Figure 2A) and this trend could also be seen for the individual ETs (Figure 2B–I).

The negative relationship between grain yield and GPC is apparent but there is a very wide spread of results, indicating the complexity of the relationship and the influence of diverse environmental factors on the yield and GPC (Figure 2A). By plotting the data for each environment type separately, more clarity in the trends emerged (Figure 2B–I). The regression line for the grain yield–GPC relationship of each ET was steepest in ET4 (Figure 2B), but close to horizontal in ET0 (Figure 2I). However, the high variation within ETs counteracted this clarity of trends, particularly, for the ETs between ET0 and ET4 (Figure 2C–H). Therefore, the slope values of the grain yield–GPC relationship were calculated for individual sites in each year to reduce the variation between sites within ETs (Table 2). This slope increased from −0.45 in ET0 to −1.63 in ET4 (Table 2) indicating that the strength of the negative yield and GPC relationship is associated with the severity of drought stress.

Table 2. Average grain yield (GY), grain protein content (GPC), and the slope of the GY–GPC relationship in individual environment types (ETs). Slope, average GY and average GPC were obtained from individual sites in each year and have been averaged. SE: standard errors.

Environment Type	ET0	ET1	ET1/2	ET2	ET2/3	ET3	ET3/4	ET4
Average GPC (%)	11.2	11.6	11.6	11.9	12.1	13.1	12.2	11.8
Average GY (t/ha)	9.2	3.3	4.3	3.1	2.9	3.2	2.6	1.6
Average slope of GY-GPC relationship	−0.45	−1.19	−0.93	−1.27	−1.43	−1.35	−1.44	−1.63
SE of GPC	0.5	0.1	0.1	0.1	0.1	0.2	0.1	0.2
SE of GY	0.6	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Range of Average GPC (%)	7	10.37	12.2	13.8	13.2	10.56	12.8	7.8
Range of Average GY (t/ha)	7.85	6.71	10.46	11.34	8.17	6.93	6.67	3.05

An even clearer relationship can be seen between the steepness of the grain yield–GPC slope and the average yield in each ET (Table 2 and Figure 3).

The variation in GPC across the range of different yielding environments was high. For example, GPC varied from 8.9% to 18.3% at sites where the average grain yield was around 2 t/ha (Figure 2A). This high GPC variation was also observed in individual ETs (Figure 2B–I).

The nature of variation in GPC was explored by examining the performance of individual varieties within the large NVT dataset. For this purpose, six varieties were selected based on their average GPC and their inclusion is a large number of trials at diverse sites (Figure 4).

Despite the large variation in the GPC of individual varieties (Figure 4), the median GPC of Spitfire was high, particularly, compared to QAL2000 and Gazelle (Figure 4A). Under similar dominant ETs (Figure 4C), the first and third quartile of the GPC varied between 11.5% and 14.8% in Spitfire, and 9.7% and 12% in QAL2000. Differences between low and high GPC varieties were even more evident with GPD values. Accordingly, the first and third quartiles of GPD was 0.3 and 1.2 in Spitfire, and −1.6 and −0.7 in QAL2000 (Figure 4D). In the entire NVT dataset from all ETs, Gazelle showed the lowest median GPD among the selected varieties (Figure 4B).
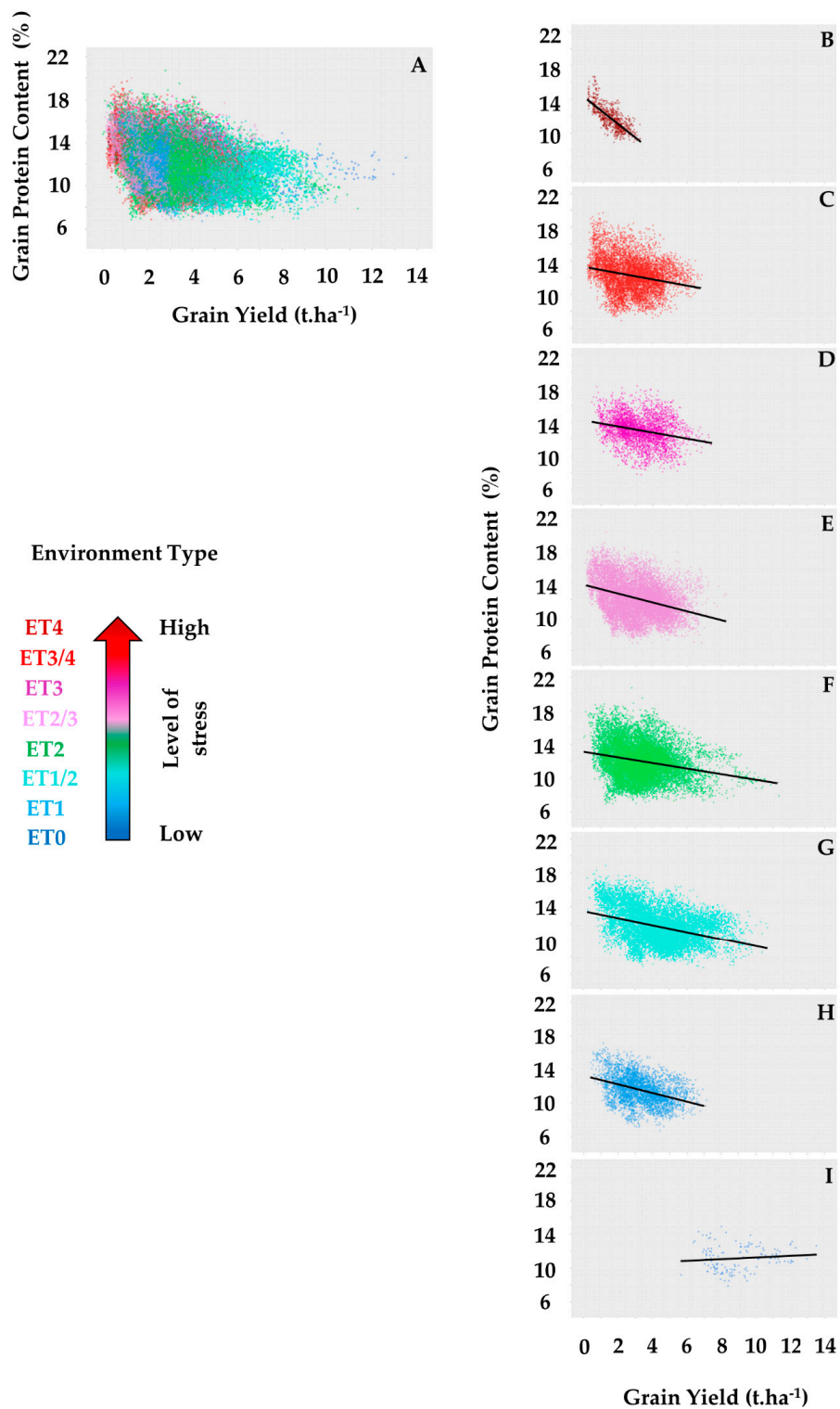
Figure 2. Relationship between grain yield and grain protein content across all (**A**) and in the individual environment types (ETs) (**B–I**). For the entire dataset (**A**), 215 wheat varieties were grown in 206 NVT sites across the Australian wheat-belt and Tasmania. The total number of varieties × site × year × season data in (**A**) were 52,822. The number of varieties, years and sites for the individual ETs in (**B–I**) are shown in Table 1. The lines in (**B–I**) show the best linear fit to the data. The negative relationships in all figures, except (I), are significant. The highest and lowest R^2 values are 0.57 and 0.01 for (B) and (I), respectively.
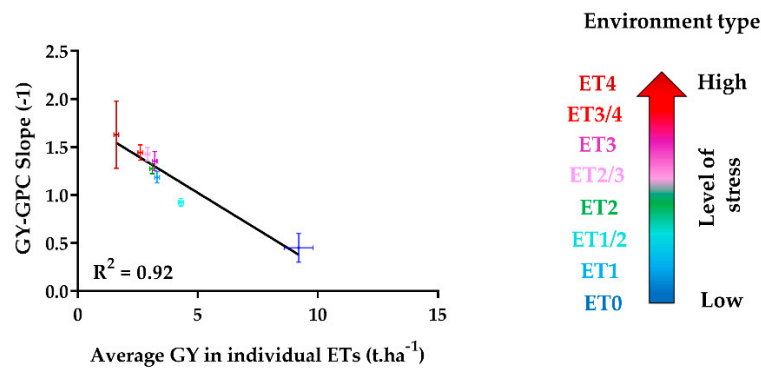
Figure 3. Relationship between the average grain yield (GY) in different environmental types (ETs), and the slope of the relationship between grain yield and grain protein content. Slope, average grain yield and average grain protein content were obtained from individual sites in each year and have been averaged. Error bars indicate the standard errors. R^2 represents the correlation coefficient.
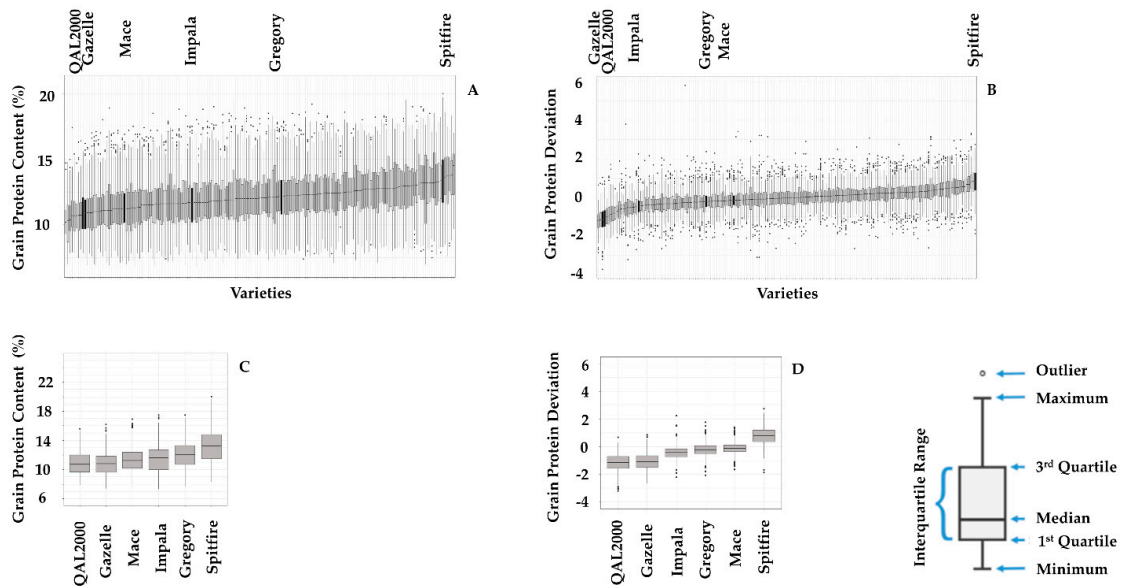

Figure 4. Grain protein content (**A**,**C**) and grain protein deviation (**B**,**D**) of 179 varieties in 206 sites, and 6 varieties in 168 sites from 2008 to 2018. ETs common between 6 varieties in (**C**,**D**) are ET1/2, ET2, ET2/3, and ET3/4. Grain protein deviation of each variety is calculated separately for each site, season, and year. The number of available trial data for each variety in (**A**,**C**) ranged from 50 to 896. The data available for the six selected varieties in (**C**,**D**) were: Spitfire: 366, Mace: 823, Gregory: 680, Impala: 429, QAL2000: 305 and Gazelle: 371. Boxes refer to the 25th (first quartile), 50th (median), and 75th (third quartile) percentile of data. (**A**,**B**) are obtained and modified from the Supplementary Figure S2 of Rahimi Eichi et al., 2019.

For the six selected varieties, the relationship between grain yield and GPC was plotted (Figure 5). All varieties showed a similar negative correlation between grain yield and GPC although they showed a large variation in GPC. There is also large variation in GPC across the yield spectrum which reflects the complexity of the influences of wheat production environments on GPC (Figure 5A–F and Supplementary Data S2).
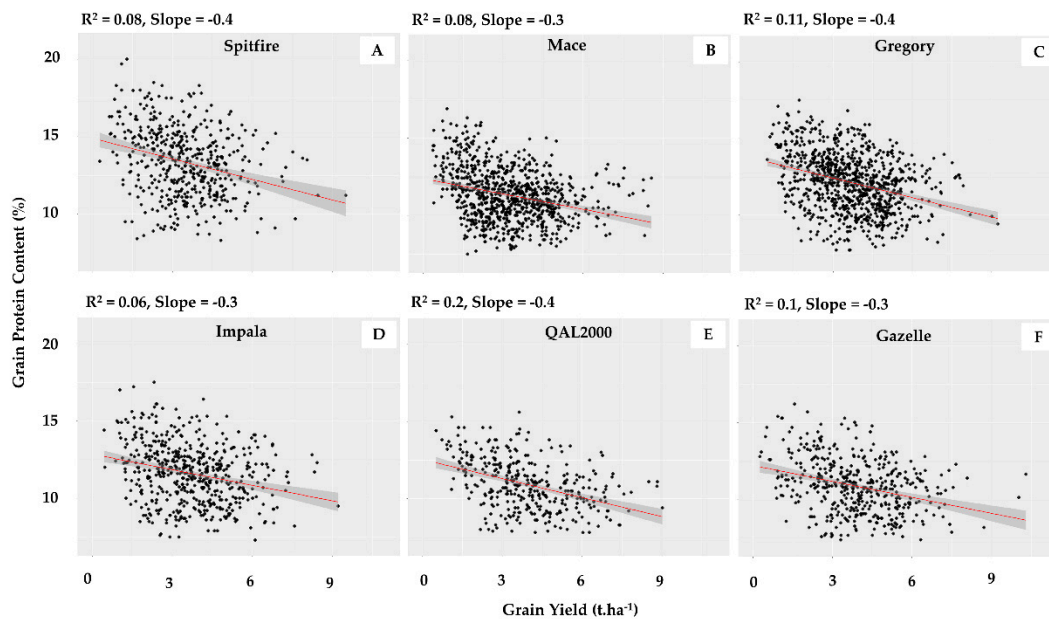
Figure 5. Relationship between grain yield and grain protein content of six selected wheat varieties obtained from NVT sites across Australia between 2008 and 2018. The number of available data for each variety was 476 (Spitfire), 850 (Mace), 897 (Gregory), 522 (Impala), 346 (QAL2000), and 445 (Gazelle). R^2 represents the correlation coefficient, and slope refers to the slope of the regression line. The negative relationship in all figures is significant.

4. Discussion

In this study, we have made use of a large, publicly available field trial dataset, to explore the relationship between stress, yield and grain protein content for wheat production in Australia. The NVT trial scheme in Australia is similar to varietal evaluation schemes operating in many countries and regions. These trials provide a potentially valuable resource for researchers since they cover a large number of trials and production environments. However, the trials are primarily designed to allow growers and agricultural agencies to compare the performance of different varieties in their regions. Consequently, the trial design does not necessarily reflect the rigor normally deployed in a scientific experiment and this can present issues with the analysis of trial data. Through our analysis of the NVT dataset, we have sought to highlight the advantages and problems in using large varietal trials.

In order to undertake this analysis, we needed to associate the 206 field sites for the NVT trials with environments. Previous studies from Chenu and Deihimfard [28], and Ababaei and Chenu [39] provided a good framework to categorize Australian wheat-belt regions based on their environmental characteristics. Yield and environment information studies indicated clear differences between environment types. Cumulative precipitation and simulated water-stress index reduced from ET1 to ET4. Based on Chenu, Deihimfard [28] work, we were able to group the trials into seven ETs. We then added the eighth environment to cover the high rainfall and high yielding sites in Tasmania. In comparison with the high rainfall zones (HRZ) of mainland Australia, Tasmania has higher rainfall and a milder climate with fewer extreme hot and cold temperatures [45]. These conditions allow average wheat yields of almost 9 t/ha in the ET0 of the NVT dataset. Accordingly, potential average yield of wheat in HRZ of Tasmania (ET0) is ~10 t/ha [45].

Some explanation is also needed for ET1, which does not follow the expected trend for yield (Figure 1A and light blue points in Figure 2A). Despite the higher rainfall and simulated water-stress index in ET1 (Supplementary Table S1), its average grain yield was less than of ET1/2 (Figure 1A). In this context, Ababaei and Chenu [39] showed lower simulated biomass and yield in ET1 compared to ET2 (Supplementary Table S1). The lower yield of Western Australia's high rainfall zone (ET1) compared to South–Eastern (ET1/2) and Tasmanian (ET0) high rainfall regions was shown in previous

studies [43,45]. Chenu, Deihimfard [28] categorized all ETs mainly based on the time and severity of drought stress regardless of their yield potential. The low yield in ET1, 50% of yield potential, might be due to the poor fertility of their cropping soils [42–44]. The shorter growing season of ET1 compared to other HRZ may also result in reduced yield [43]. A previous study on grain yield–GPC relationship suggested the separate assessment of Western Australian sites from other regions for GPC improvement in breeding programs [20]. The average yield of ET1 in this study was 3.3 t/ha, while the yield in Western HRZ was 2.7 t/ha [43,44]. The slightly higher average yield of NVT compared to the actual yield in Western HRZ can be due to the agronomic practices in NVT sites.

The higher average of GPC in ET3 compared to other environments (Figure 1B) might be due to differences in the drought pattern. It has been previously shown that drought stress before the end of grain filling increases GPC in wheat [46]. N uptake after anthesis, which increases N accumulation into grains, depends on the availability of water and nitrogen [35,47,48]. NVT sites receive adequate N fertilizer, therefore water availability may be the limiting factor of N uptake [24]. Relief from drought stress during grain filling in ET3 may permit post-anthesis N uptake. In ET4, however, water stress begins early in the season and intensifies towards the end of grain filling [28]. Consequently, pre and post-anthesis N uptake may not be sufficient to maintain yield and GPC in ET4. The high trade-off between yield and GPC (Figure 2B,D and Table 2) can be another reason for the lower GPC in ET4 compared to ET3. In ET0, on the other hand, the dilution effect of high grain yield might reduce GPC in comparison with ET3.

There is the potential for a range of variables to affect yield and GPC data from the NVT trials, including varieties, seasonal factors, level of replication, edaphic and disease pressures, and agronomic practices. In order to assess the reliability of the ET effects on yield and GPC, six varieties were examined and compared to the full dataset (Figures 4 and 5). The same varieties were used previously to examine their responses to N application in a controlled environment where plant growth rates were assessed [33]. In the previous study, it was found that the severity of stress, in that case the stress was induced through N starvation, intensified the negative relationship between yield and GPC. Grain protein content in wheat is strongly influenced by environment, and environmental factors have a greater impact than genetics effect [49,50].

The agronomic management of the NVT system aims to reflect the optimum practices for all sites and varies between sites. Consequently, N fertilizer may be applied at different rates between ETs. This may add to the variability of yield and GPC both between and within ETs. Moreover, in this study sites with similar first dominant ET were categorized together regardless of their second, third, or fourth dominant ETs. Therefore, the high yield and GPC variation of individual ETs (Figures 1 and 2) might come from inconsistencies in the ETs classification or different agronomic practices. For instance, the second dominant ET of two ET2 sites, Birchip and Cummins, were ET4 and ET1/2, respectively. Therefore, the average yield at Birchip was 3 t/ha, whereas, it was 4.9 t/ha for Cummins. However, these comparisons are based on averages and only indicate trends.

The inverse relationship between yield and GPC was stronger in low than in high yielding ETs (Figure 2A–I and Table 2). The NVT data show that a 1 t/ha increase in yield leads to an average GPC loss of 0.45% and 1.63% in ET0 and ET4, respectively. Previous studies on wheat also found a stronger negative relationship between yield and GPC under low N compared to high N supply [20,33,51]. The strong GPC-yield trade off under low N conditions can be due to a higher priority for the plants to maintain grain number and weight than for grain protein accumulation. A study on wheat [33] showed that under low N, high GPC varieties bred for low yielding environments sacrificed biomass and, consequently, yield in order to reserve N for grain protein. Conversely, low GPC varieties selected for high yielding regions used the available N primarily for biomass and yield regardless of grain N concentration. Reducing biomass and yield in favor of GPC increase can be considered as a strategy for plants to maintain GPC under stress. Under the stressed scenarios, fewer grains are produced but with sufficient nutrient levels to support germination and plant establishment [52–54]. The steep slope

of the yield–GPC relationship under stress suggests that simultaneous selection for high yield and GPC will be more effective in low than in high yielding regions.

Until the 1930s, Australian wheat production was dominated by soft and low GPC genotypes. However, since the 1960s the proportion of hard varieties with high GPC began to increase. Hard and soft wheat varieties were segregated between the 1950s and 1970s in different Australian regions. This led to the payment of premiums for high GPC hard wheats [50], and soft and feed varieties that were high yielding but with low GPC stayed in irrigated and high rainfall regions [50,55,56]. Higher GPC varieties, tended to be grown in dryer regions with lower yield potentials and the majority of wheat produced in Australia is grown in low yielding regions [50,55,57]. Over the last decade, there has been a significant increase in the premium price for high GPC wheat grains. Consequently, growers and breeders have targeted high GPC varieties even though this results in a small yield penalty [21,22].

Corresponding to the work of Bogart et al., [15], this study also showed the relative robustness of GPD across different environments. GPD corrects for the environmental effects on GPC and reveals the genotypic differences more clearly. The large variation in GPC due to environmental effects were reduced using GPD (Figure 4). The varieties Gazelle and QAL2000, which are considered as low GPC soft varieties had the lowest GPD, while, Spitfire, Mace, and Impala, high GPC varieties, showed higher average GPD. Therefore, GPD can be used as a potential target for selection in wheat breeding.

Our results (Figure 5 and Supplementary Data S2) confirmed a previous study on NVT data [21], indicating the inverse relationship between yield and GPC in individual varieties. However, this negative relationship was as small as a 1 t/ha increase in yield corresponded to the average GPC loss of ~0.4%. Indeed, the negative relationship between yield and GPC could be managed by applying suitable agronomic practices [9,51,58]. As mentioned above, the agronomic practices in NVT sites are based on the optimum for that region. For instance, delaying the final fertilizer application to around heading has been shown to increase GPC without yield penalty [15,59]. However, the success of this approach depends on climate conditions, particularly, water availability after anthesis. In the absence of sufficient water, the additional N is not beneficial and leads to multiple environmental consequences such as underground water pollution and eutrophication.

In the NVT system, varieties are selected to suit the production environments. Therefore, varieties, agronomic practices, and the number of sites and entries can vary between years, sites, and ETs. This variability means that statistical tests need to be applied with caution. The unbalanced nature of these trials can affect the validity of statistical tests, which often assume that varieties have been allocated at random to trials. However, in the NVT system the variables such as varieties and agronomical treatments (e.g., sowing date, fertilizer rate, chemical application, etc.) are not random but systematically decided in each field site. For example, the variety list of each NVT site is selected based on their suitability to the region [23]. If a variety does not produce acceptable yield or GPC in a site, it will usually be eliminated from the list at that site. Such limitations lead to the sampling process bias and, consequently, reduce the value of statistical assumptions. Accordingly, in this study, comparisons between varieties and ETs were based on averages or medians and only indicate trends. Showing the trends of values for different ETs and varieties indicates the potential of using such large numbers of data for multi-environmental studies. However, these are unbalanced datasets and this limits there value for detailed statistical analysis. A possible approach to analyze such unbalanced data can be the method developed by Smith and Cullis [60]. In other words, this study presented a useful dataset that others can examine further in more statistically rigorous ways to answer questions about the relationship between yield and GPC. Applying one standard agronomic practice in all sites could also improve the comprehensive multi-environmental analysis of NVT dataset in future.

5. Conclusions

While grain yield is of critical importance to wheat farmers, the protein content will influence the value of the grain. The relationship between grain yield and protein content is complex and highly dependent on the production environment but breeders have targeted both traits for selection in their

breeding programs. Achieving significant genetic gain for both yield and protein is difficult, given the large environmental and relatively small genetic component. Controlled environment studies have been helpful in understanding the impact of stress on the yield/protein relationship [11,20,33] but these results require validation through actual field trials. Here we have explored the option of exploiting the extensive field trial data used to evaluate germplasm across a large number of environments. Many countries run extensive variety evaluation trials, and these usually cover a large number of sites and environments. However, the trials are not designed with a view for the detailed statistical analysis required to explore the genotype × environment interactions that strongly influence the yield/protein relationship. In this study, we have attempted this analysis by using the ETs, categorized in Chenu and Deihimfard [28] and based on the time and intensity of drought stress, identified the potential for robust analysis of the impact of drought on yield–GPC relationship. However, further improvements in statistical approaches and NVT trials management may enhance the precision of the analysis.

The negative relationship between yield and GPC is more significant in low than in high yielding environments. This conclusion is consistent with controlled environment studies [11,20,33] and supports the view that GPC needs to be interpreted with a consideration of environmental factors that may limit yield. Selecting wheat varieties in low yielding regions might be more effective for the simultaneous increase of yield and GPC, and transferring varieties bred in low yielding environments to high yielding regions might provide results better than the reverse.

GPD revealed the genetic differences across different ETs, and therefore can be considered as a worthwhile potential target for breeders to improve both yield and GPC in wheat. Despite the genetic differences between varieties, selecting suitable sites and agronomic practices can help wheat farmers to achieve their targeted GPC [55,61,62]. For farmers, GPC loss with yield increase is best compensated using suitable region and agronomic management. Breeders, on the other hand, can select for varieties in environments where the impact of stress on GPC loss can be assessed.

ET3 regions might be suitable to grow high GPC varieties with reasonable yield while the high rainfall regions of Tasmania and the South–East Australian mainland should remain focused on soft or feed varieties with high yield but low GPC.

Author Contributions: Conceptualization, V.R.E., P.L., T.G., and M.O.; methodology, V.R.E., P.L., T.G., M.O., P.E., and M.R.; formal analysis, V.R.E. and B.D.; investigation, V.R.E.; writing—original draft preparation, V.R.E., T.G., P.L., and M.O.; writing—review and editing, V.R.E, T.G., P.L, and M.O.; supervision, P.L., T.G., and M.O. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Shewry, P.R.; Hey, S.J. The contribution of wheat to human diet and health. *Food Energy Secur.* **2015**, *4*, 178–202. [CrossRef]

2. Curtis, T.; Halford, N.G. Food security: The challenge of increasing wheat yield and the importance of not compromising food safety. *Ann. Appl. Boil.* **2014**, *164*, 354–372. [CrossRef]

3. Godfray, H.C.J.; Beddington, J.R.; Crute, I.R.; Haddad, L.; Lawrence, D.; Muir, J.F.; Pretty, J.; Robinson, S.; Thomas, S.M.; Toulmin, C. Food security: The challenge of feeding 9 billion people. *Science* **2010**, *327*, 812–818. [CrossRef]

4. FAOSTAT. Available online: http://faostat.fao.org/default.aspx (accessed on 20 February 2020).

5. FAO. *How to Feed the World 2050*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2009.

6. Pardey, P.; Beddow, J.; Hurley, T.; Beatty, T.; Eidman, V. A bounds analysis of world food futures: Global agriculture through to 2050. *Aust. J. Agric. Resour. Econ.* **2014**, *58*, 571–589. [CrossRef]

7. Husenov, B.; Makhkamov, M.; Garkava-Gustavsson, L.; Muminjanov, H.; Johansson, E. Breeding for wheat quality to assure food security of a staple crop: The case study of Tajikistan. *Agric. Food Secur.* **2015**, *4*, 9. [CrossRef]

8. Kuhn, J.C.; Stubbs, T.L.; Carter, A.H. Effect of the gpc-b1 allele in hard red winter wheat in the US pacific northwest. *Crop. Sci.* **2016**, *56*, 1009–1017. [CrossRef]

9. Laidig, F.; Piepho, H.P.; Rentel, D.; Drobek, T.; Meyer, U.; Huesken, A. Breeding progress, environmental variation and correlation of winter wheat yield and quality traits in german official variety trials and on-farm during 1983–2014. *Theor. Appl. Genet.* **2017**, *130*, 223–245. [CrossRef]

10. Blakeney, A.B.; Cracknell, R.L.; Crosbie, G.B.; Jefferies, S.P.; Miskelly, D.M.; O'Brien, L.; Panozzo, J.F.; Suter, D.A.I.; Solah, V.; Watts, T.; et al. *Understanding Australian Wheat Quality: A Basic Introduction to Australian Wheat Quality*; GRDC: Canberra, Australia, 2009.

11. Oury, F.X.; Bérard, P.; Brancourt-Hulmel, M.; Depatureaux, C.; Doussinault, G.; Galic, N.; Giraud, A.; Heumez, E.; Lecomte, C.; Pluchard, P.; et al. Yield and grain protein concentration in bread wheat: A review and a study of multi-annual data from a french breeding program. *J. Genet. Breed.* **2003**, *57*, 59–68.

12. Cooper, M.; Woodruff, D.R.; Philips, I.G.; Basford, K.E.; Gilmour, A.R. Genotype-by-management interactions for grain yield and grain protein concentration of wheat. *Field Crop. Res.* **2001**, *70*, 87–88. [CrossRef]

13. Zörb, C.; Ludewig, U.; Hawkesford, M.J. Perspective on wheat yield and quality with reduced nitrogen supply. *Trends Plant Sci.* **2018**, *23*, 1029–1037. [CrossRef]

14. Monaghan, J.; Snape, J.; Chojecki, A.; Kettlewell, P. The use of grain protein deviation for identifying wheat cultivars with high grain protein concentration and yield. *Int. J. Plant Breed.* **2001**, *122*, 309–317.

15. Bogard, M.; Allard, V.; Brancourt-Hulmel, M.; Heumez, E.; Machet, J.-M.; Jeuffroy, M.-H.; Gate, P.; Martre, P.; Le Gouis, J. Deviation from the grain protein concentration–grain yield negative relationship is highly correlated to post-anthesis n uptake in winter wheat. *J. Exp. Bot.* **2010**, *61*, 4303–4312. [CrossRef] [PubMed]

16. Michel, S.; Loschenberger, F.; Ametz, C.; Pachler, B.; Sparry, E.; Burstmayr, H. Simultaneous selection for grain yield and protein content in genomics-assisted wheat breeding. *Theor. Appl. Genet.* **2019**, *132*, 1745–1760. [CrossRef] [PubMed]

17. Bilgin, O.; Guzman, C.; Baser, I.; Crossa, J.; Korkut, K.Z. Evaluation of grain yield and quality traits of bread wheat genotypes cultivated in northwest turkey. *Crop Sci.* **2016**, *56*, 73–84. [CrossRef]

18. Thorwarth, P.; Piepho, H.P.; Zhao, Y.; Ebmeyer, E.; Schacht, J.; Schachschneider, R.; Kazman, E.; Reif, J.C.; Würschum, T.; Longin, C.F.H. Higher grain yield and higher grain protein deviation underline the potential of hybrid wheat for a sustainable agriculture. *Plant Breed.* **2018**, *137*, 326–337. [CrossRef]

19. Oury, F.X.; Godin, C. Yield and grain protein concentration in bread wheat: How to use the negative relationship between the two characters to identify favourable genotypes? *Euphytica* **2007**, *157*, 45–57. [CrossRef]

20. Mahjourimajd, S.; Taylor, J.; Rengel, Z.; Khabaz-Saberi, H.; Kuchel, H.; Okamoto, M.; Langridge, P. The genetic control of grain protein content under variable nitrogen supply in an australian wheat mapping population. *PLoS ONE* **2016**, *11*, e0159371. [CrossRef]

21. Brill, R.; Gardner, M.; Fettell, N.; Martin, P.; Haskins, B.; McMullen, G. Grain Protein Concentration of Several Commercial Wheat Varieties. In Proceedings of the 16th ASA Conference: Capturing Opportunities and Overcoming Obstacles in Australian Agronomy, Armidale, Australia, 14–18 October 2012.

22. Fettell, N.; Brill, R.; Gardner, M.; McMullen, G. *Yield and Protein Relationships in Wheat*; GRDC: Canberra, Australia, 2012.

23. Giles, T. How nvt works. In *National Variety Trials Supplement*; Leonard, E., Ed.; GRDC: Canberra, Australia, 2012; p. 3.

24. Giles, T.; Bedggood, A.; Kelly, A. Trials with maximum value. In *National Variety Trials Supplement*; Leonard, E., Ed.; GRDC: Canberra, Australia, 2012.

25. Hochman, Z. Pushing the yield frontier. In *National Variety Trials Supplement*; Leonard, E., Ed.; GRDC: Canberra, Australia, 2012; p. 13.

26. Braun, H.J.; Rajaram, S.; vanGinkel, M. Cimmyt's approach to breeding for wide adaptation. *Euphytica* **1996**, *92*, 175–183. [CrossRef]

27. Hodson, D.P.; White, J.W. Use of spatial analyses for global characterization of wheat-based production systems. *J. Agric. Sci.* **2007**, *145*, 115–125. [CrossRef]

28. Chenu, K.; Deihimfard, R.; Chapman, S.C. Large-scale characterization of drought pattern: A continent-wide modelling approach applied to the australian wheatbelt spatial and temporal trends. *New Phytol.* **2013**, *198*, 801–820. [CrossRef]

29. Reynolds, M.; Dreccer, F.; Trethowan, R. Drought-adaptive traits derived from wheat wild relatives and landraces. *J. Exp. Bot.* **2007**, *58*, 177–186. [CrossRef] [PubMed]

30. Dupont, F.M.; Hurkman, W.J.; Vensel, W.H.; Tanaka, C.; Kothari, K.M.; Chung, O.K.; Altenbach, S.B. Protein accumulation and composition in wheat grains: Effects of mineral nutrients and high temperature. *Eur. J. Agron.* **2006**, *25*, 96–107. [CrossRef]

31. Flagella, Z.; Giuliani, M.M.; Giuzio, L.; Volpi, C.; Masci, S. Influence of water deficit on durum wheat storage protein composition and technological quality. *Eur. J. Agron.* **2010**, *33*, 197–207. [CrossRef]

32. Rharrabti, Y.; Villegas, D.; Royo, C.; Martos-Nunez, V.; del Moral, L.F.G. Durum wheat quality in mediterranean environments ii. Influence of climatic variables and relationships between quality parameters. *Field Crop. Res.* **2003**, *80*, 133–140. [CrossRef]

33. Rahimi Eichi, V.; Okamato, M.; Haefele, S.M.; Jewell, N.; Brien, C.; Garnett, T.; Langridge, P. Understanding the interactions between biomass, grain production and grain protein content in high and low protein wheat genotypes under controlled environments. *Agronomy* **2019**, *9*, 706. [CrossRef]

34. Sadras, V.O.; McDonald, G. *Benchmarking Wheat Water Use Efficiency: Accounting for Climate and Nitrogen*; GRDC: Adelaide, Australia, 2012.

35. Stoddard, F.L.; Marshall, D.R. Variability in grain protein in australian hexaploid wheats. *Aust. J. Agric. Res.* **1990**, *41*, 277–288. [CrossRef]

36. Smith, A.B.; Thompson, R.; Butler, D.G.; Cullis, B.R. The design and analysis of variety trials using mixtures of composite and individual plot samples. *J. R. Stat. Soc. Ser. C (Appl. Stat.)* **2011**, *60*, 437–455. [CrossRef]

37. Chapman, S.C.; Hammer, G.L.; Meinke, H. A sunflower simulation model: I. *Model development. Agron. J.* **1993**, *85*, 725–735. [CrossRef]

38. Chenu, K.; Cooper, M.; Hammer, G.L.; Mathews, K.L.; Dreccer, M.F.; Chapman, S.C. Environment characterization as an aid to wheat improvement: Interpreting genotype–environment interactions by modelling water-deficit patterns in north-eastern australia. *J. Exp. Bot.* **2011**, *62*, 1743–1755. [CrossRef]

39. Ababaei, B.; Chenu, K. Heat shocks increasingly impede grain filling but have little effect on grain setting across the Australian wheatbelt. *Agric. For. Meteorol.* **2020**, *284*, 107889. [CrossRef]

40. R Foundation for Statistical Computing. *R: A Language and Environment for Statistical Computing*; R Development Core Team: Vienna, Austria, 2009.

41. Bureau of Meteorology. *Climate Statistics for Australian Locations, Monthly Climate Statistics, Summary Statistics*, 29th ed.; Australian Government: Launceston, Ti Tree Bend, Australia, 2019.

42. Acuna, T.B.; Dean, G.; Riffkin, P. Constraints to achieving high potential yield of wheat in a temperate, high-rainfall environment in south-eastern Australia. *Crop Pasture Sci.* **2011**, *62*, 125–136. [CrossRef]

43. Zhang, H.; Turner, N.C.; Poole, M.L.; Simpson, N. Crop production in the high rainfall zones of Southern Australia—Potential, constraints and opportunities. *Aust. J. Exp. Agric.* **2006**, *46*, 1035–1049. [CrossRef]

44. Chen, W.; Bell, R.W.; Brennan, R.F.; Bowden, J.W.; Dobermann, A.; Rengel, Z.; Porter, W. Key crop nutrient management issues in the western australia grains industry: A review. *Aust. J. Soil Res.* **2009**, *47*, 1–18. [CrossRef]

45. Hill, N.; Zhang, H.; Trezise, T.; Young, J.; Moyes, N.; Carslake, L.; Turner, N.C.; Anderson, W.; Poole, M. *Successful Cropping in the High Rainfall Zone of Western Australia: Crop Research and Extension in the Zone*; Department of Agriculture and Food: Perth, Western Australia, 2005.

46. Gooding, M.J.; Ellis, R.H.; Shewry, P.R.; Schofield, J.D. Effects of restricted water availability and increasedtemperature on the grain filling, dryingand quality of winter wheat. *J. Cereal Sci.* **2003**, *37*, 295–309. [CrossRef]

47. Barbottin, A.; Lecomte, C.; Bouchard, C.; Jeuffroy, M.H. Nitrogen remobilization during grain filling in wheat: Genotypic and environmental effects. *Crop Sci.* **2005**, *45*, 1141–1150. [CrossRef]

48. Triboi, E.; Martre, P.; Triboi-Blondel, A.M. Environmentally-induced changes in protein composition in developing grains of wheat are related to changes in total protein content. *J. Exp. Bot.* **2003**, *54*, 1731–1742. [CrossRef]

49. Crosbie, G.B.; Fisher, H. Variation in wheat protein content: The effect of environment. *J. Agric. West. Aust.* **1987**, *28*, 124–127.

50. Simmonds, D.H. *Wheat and Wheat Quality in Australia*; CSIRO Publishing: Clayton, Australia, 1989.

51. Torrion, J.A.; Walsh, O.S.; Liang, X.; Bicego, B.; Sapkota, A. Managing 'egan' wheat with a gene for high grain protein. *Agrosystems Geosci. Environ.* **2019**, *2*, 1–8. [CrossRef]

52. Zorb, C.; Becker, E.; Merkt, N.; Kafka, S.; Schmidt, S.; Schmidhalter, U. Shift of grain protein composition in bread wheat under summer drought events. *J. Plant Nutr. Soil Sci.* **2017**, *180*, 49–55. [CrossRef]

53. Stone, P.J.; Nicolas, M.E. A survey of the effects of high-temperature during grain filling on yield and quality of 75 wheat cultivars. *Aust. J. Agric. Res.* **1995**, *46*, 475–492. [CrossRef]

54. Daniel, C.; Triboi, E. Changes in wheat protein aggregation during grain development: Effects of temperatures and water stress. *Eur. J. Agron.* **2002**, *16*, 1–12. [CrossRef]

55. Anderson, W.K.; Sawkins, D. Production practices for improved grain yield and quality of soft wheats in western australia. *Aust. J. Exp. Agric.* **1997**, *37*, 173–180. [CrossRef]

56. Spragg, J. *Benefit to Australian Grain Growers in the Feed Grain Market*; Grains Research and Development Corporation: Victoria, Australia, 2008.

57. Turner, N.C. Agronomic options for improving rainfall-use efficiency of crops in dryland farming systems. *J. Exp. Bot.* **2004**, *55*, 2413–2425. [CrossRef] [PubMed]

58. Rozbicki, J.; Ceglinska, A.; Gozdowski, D.; Jakubczak, M.; Cacak-Pietrzak, G.; Madry, W.; Golba, J.; Piechocinski, M.; Sobczynski, G.; Studnicki, M.; et al. Influence of the cultivar, environment and management on the grain yield and bread-making quality in winter wheat. *J. Cereal Sci.* **2015**, *61*, 126–132. [CrossRef]

59. Fischer, R.A.; Howe, G.N.; Ibrahim, Z. Irrigated spring wheat and timing and amount of nitrogen-fertilizer. 1. Grain-yield and protein-content. *Field Crop. Res.* **1993**, *33*, 37–56.

60. Smith, A.B.; Cullis, B.R. Plant breeding selection tools built on factor analytic mixed models for multi-environment trial data. *Euphytica* **2018**, *214*, 143. [CrossRef]

61. Anderson, W.K.; Hamza, M.A.; Sharma, D.L.; D'Antuono, M.F.; Hoyle, F.C.; Hill, N.; Shackley, B.J.; Amjad, M.; Zaicou-Kunesch, C. The role of management in yield improvement of the wheat crop—A review with special emphasis on western australia. *Aust. J. Agric. Res.* **2005**, *56*, 1137–1149. [CrossRef]

62. Anderson, W.K.; Impiglia, A. Management of dryland wheat. In *Bread Wheat Improvement and Production*; Curtis, B.C., Rajaram, S., Gomez Macpherson, H., Eds.; FAO: Rome, Italy, 2002; pp. 407–432.