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Genetic Variation in Grain Yield and Quality Traits of Spring Malting Barley

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Abstract: Cultivation of malting barley is particularly challenging as the requirements of growers, for high yield, and that of the brewing industry, seeking a specific quality criteria, must be met simultaneously. Furthermore, significant genotypic and environmental variations in grain yield and quality properties may occur. To investigate the relationships between grain yield and quality parameters of spring malting barley, a 2-year experiment was carried out in order to characterise the genotypic and year effects on grain yield, quality properties, and yield components of 23 high-yielding varieties of spring malting barley under optimal nitrogen (N) fertilisation. Compared to the grain quality properties of the grain protein content and the grain retention fraction of grain size >2.5 mm, less genotypic and environmental variation in grain yield was observed. Grain yield was closely related to spikes per m^2, suggesting the importance of tiller formation and establishment as a decisive factor influencing malting barley yields. A major interactive effect of genotypes and year on grain size was observed. Regarding weather effects, the global radiation intensity during the post-anthesis phase was the major factor affecting the final grain size in this study. Grain protein content was primarily dependent on the year effect, suggesting that optimal N fertilisation levels must vary between years to ensure the correct protein content required for the needs of the brewing industry is met. Therefore, we recommend further development strategies addressing N fertilisation and soil N mineralisation to optimise the production of spring malting barley.

Keywords: fertilizer management; grain number; grain retention; grain weight; malting quality; nitrogen fertilization; spring barley; year effect; yield

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1. Introduction

Barley is unique among crop plants and is of tremendous importance to agriculture; it is the fourth most important cereal crop in the world, after maize, rice, and wheat [1], and in Europe, approximately 12 million ha are cultivated with barley [2]. Barley grain, in the form of malt, is a perfect nutritional source of yeast, which is very important for the brewing industry. In Germany in 2020, approximately 75% of the harvested spring barley grain was delivered as malting barley [3]. In addition to achieving high yields, specific quality criteria for malting barley must be met to optimise industrial processes; barley grains are considered suitable for the malting and brewing industry if they have a protein content between 9.5% and 11.5% of dry weight, and if more than 90% of the harvested grains (i.e., the grain retention fraction) are larger than 2.5 mm [4]. The price paid to farmers is much lower for grains not meeting these requirements, which can be downgraded to feed barley [3]. However, the relationship between grain yield, grain size, and protein content is generally negative [5]. To meet the malt quality requirements, therefore, grain yield and quality parameters of malting barley must be balanced. Furthermore, there has been a significant amount of work done to evaluate the factors influencing the malt barley yield and quality including different varieties, environmental conditions and agronomic practices (e.g., [6–12]). Molina-Cano et al. [6], based on 11 studies, showed contradictory results regarding genotype × environment interaction, and concluded that generally, the environmental effects had more influence on total variation than their interaction with genotypes. Therefore, it is necessary to evaluate the genetic variation of yield and quality traits of regionally available varieties, as they will be influenced by environmental conditions and the relationships between yield and quality traits.

The yield of small grain cereals is a product of two components: grain number and grain weight. Previous studies have demonstrated that while grain number in barley varies widely with location and season and typically accounts for the majority of the variation in yield across environments [10,13–19], the mean grain weight and size tend to be less variable and poorly correlated with yield [10,12–14,20]. Because large grains provide a greater malt extract potential for the beer industry, grain size is an important parameter for malting quality, and is closely associated with the mean grain weight [16]. Although grain weight is often considered to be the most stable yield component of barley [13], significant genotypic and environmental variation can occur [7–9].

It is known that, during the pre-anthesis period, the number of grains per spike and the number of spikes per m^2 is determined [21,22]. In contrast, it has been generally accepted that grain weight and size are primarily determined during the post-anthesis period [16,23,24]. Grain weight and grain size were positively associated with the amount of radiation intercepted per grain during the stage of grain filling in the post-anthesis period [25,26] but were reduced by drought and heat stress [27–29]. Water stress during the grain filling period has a negative effect on barley grain weight and size, primarily due to a reduction in the grain filling duration [30–33], and low radiation likely reduces the grain filling rate [16,17,26,31,34]. The climate in the investigated region is also characterized by variability in the incident radiation over different years during the summer months [29,35], and thus, the natural occurrence of such an effect on the grain size of malting barley during post-anthesis is plausible. In particular, climate change in the past years has led to more frequent heat spells and droughts during summer [29,35]. In order to ensure yield stability and quality under variable weather conditions, a better understanding of the control of grain number and size and their response to genetic variation between years will play a crucial role in determining the possible limits to yield and quality of malting barley. Since the weather in different years varies greatly, we considered the year effect as the environmental effect in this study.

The grain protein concentration in cereal crops is dependent on a multitude of factors, including N supply, N uptake before anthesis, remobilization to the grain during grain filling [11,36–38], environmental conditions such as temperature and water stress [28,39], and genotype × environmental interactions [40,41]. Many of the above factors make it difficult for malting barley growers to control or implement practical methods to produce grain fulfilling the correct end-use specification. For spring malting barley grown in southern Germany, the Bavarian State Research Center for Agriculture (LfL) has recommended 120–140 kg N ha^{-1} for spring malting barley. However, little research has been conducted on how years varying in air temperature, radiation, and precipitation affect the genotypic variation in grain yield, quality character, and yield components of spring malting barley, or what the relationships between yield and yield components under the recommended N fertilisation are.

Therefore, the aim of this study was to characterise the genotypic variation in grain yield, quality properties, and yield components which contribute to variations in grain yield and quality from 23 spring malting barley varieties in a two-year experiment.

2. Materials and Methods

2.1. Field Experiments

A two-year field experiment with 23 spring malting barley cultivars in 2014 and 2015 (Table 1) was conducted at the experimental station of the Technical University of Munich at Dürnast in Germany (11°41′60″ E, 48°23′60″ N). Barley seeds were sown at mid-March at a seed density of 330 seeds m^{-2}, and the final harvest was carried out at the end of

July. A randomized block design with four replicates was used for the experiments. Plots consisted of 12 rows and were 10.9 m in length (16.35 m^2). The soil was characterized as a mostly homogeneous Cambisol of silty clay loam. Residual soil mineral nitrogen (N-min) at 60 cm depth, before sowing, was 65 kg ha^{-1} in 2014 and 40 kg ha^{-1} in 2015, respectively. Based on the local N recommendation, N fertilisation was applied as a dressing at 70 kg N ha^{-1} at sowing in both years; therefore, the total N supply was 135 kg ha^{-1} in 2014 and 110 kg ha^{-1} in 2015.

Table 1. Spring malting barley varieties in 2014 and 2015 showing name, year of release, and country of origin (G-Germany, Aus-Australia).

No.	Variety Name	Listed	Country
1	Aspen	1999	G
2	Barke	1996	G
3	Baronesse	1989	G
4	Braemar	2002	G
5	Carina	1973	G
6	Grace	2008	G
7	IPZ 24727	-	G
8	Irina	2012	G
9	Mackay	2003	AUS
10	Marthe	2005	G
11	Melius	2012	G
12	Power	1998	G
13	Quench	2006	G
14	Salome	2011	G
15	Scarlett	1995	G
16	Shakira	2004	G
17	Sissy	1990	G
18	Solist	2012	G
19	Trumpf	2003	G
20	Union	1950	G
21	Ursa	2002	G
22	Volla	1957	G
23	Wiebke	1998	G

The average annual precipitation in this region was ∼800 mm, and the average annual temperature was 7.8 °C.

2.2. Measurements and Analysis

Growth stages such as anthesis, dough ripening, and maturity stages among barley varieties were recorded (Figure 1). Plant height at the dough ripening stage was determined by an ultrasonic sensor (SICK, Waldkirch, Germany) according to Barmeier et al. [42]. Briefly, the sensor was mounted in front of the PhenoTrac IV [42] and the sensor outputs were linked and synchronised to the GPS coordinates from a Trimble RTK-GPS. Calibration of the sensors was conducted on a bare plot. The sensor boom was held at a height of ~1 m above the plants, and the driving speed was 3.5 km h^{-1}. The data output comprised of ~25 measurements across the 6 m plot length, and then average values of all the measurements per plot were calculated.

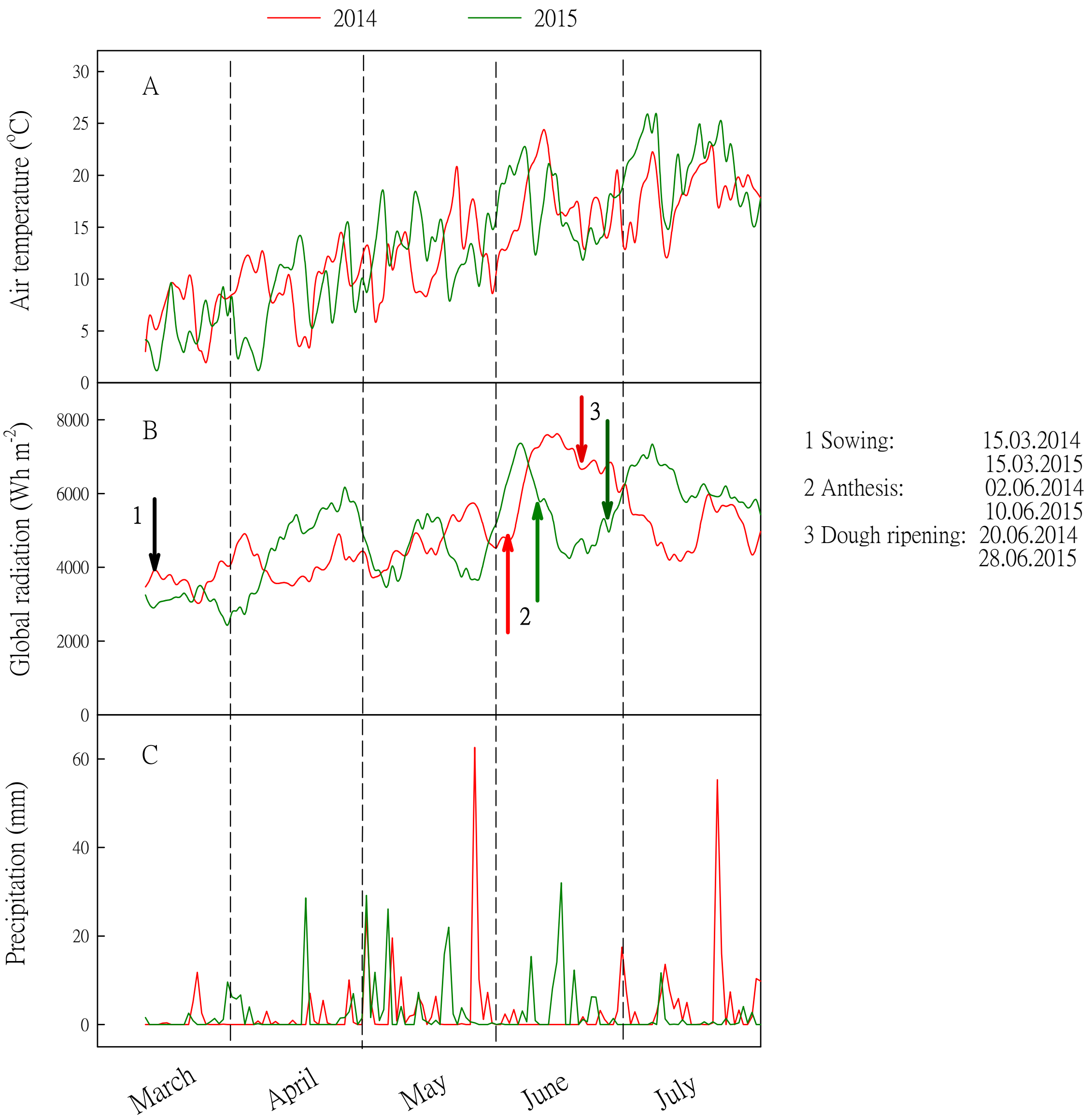

Figure 1. Daily air temperature (**A**), global radiation (**B**), and precipitation (**C**) during the growing season from March to July in 2014 and 2015 (DWD, https://www.dwd.de/, accessed on 30 July 2018). Global radiation is presented as smoothed to a 10-day moving average.

At plant maturity, the spike density (i.e., the spike number per m^2) was manually counted in 3 rows with a length of 1.5 m in each plot, and 30 plants from each plot were subsequently randomly harvested by hand-cutting. The plants were separated into ears, leaves, sheaths, and stems. The barley ears were threshed into grains and chaff. The plant material was oven-dried at 60 °C for 2 days to achieve a constant moisture content, and the dry weight was subsequently determined. The straw weight was the sum of the chaff, leaves, sheaths, and stems. The grain and straw yield (kg ha^{-1}) was calculated based on the plant density, and the harvest index (HI) was calculated based on the ratio of grain dry weight to straw dry weight. The whole plot grain yield was obtained from a combine harvester at grain maturity, which showed the comparative grain yields harvested by hand.

Grain retention fraction at grains >2.5 mm and thousand grain weight (TGW) were determined using a grain size counter (Pfeuffer Contador, Waidenburg, Germany). Grains per m^2 were estimated based on the grain yield per m^2 and TGW. Grains per spike were further calculated based on both grains and spikes per m^2.

The N content of the grains was detected by mass spectrometry using an Isotope Radio Mass Spectrometer with an ANCA SL 20-20 preparation unit (Europa Scientific, Crewe, UK). A factor of 6.25 for the N content of grain on a dry matter basis was used to estimate the raw protein content of barley grains.

2.3. Statistical Analysis

Trials were conducted as a randomized complete block design (RCBD) with 4 replicate blocks and conducted over 2 years. To quantify and compare effect sizes, ANOVA over both years was conducted with the model:

$$V + Y + V{:}Y + B + V{:}B, \qquad (1)$$

where V is variety main effect, Y is the year main effect, V:Y is the variety x year interaction, B is the block main effect, and V:B is the replicate block effect nested within years. All effects were taken as fixed, as we were interested in the variety performance in the particular years.

Variety means and their standard errors within years were calculated with the model:

$$V + B \qquad (2)$$

Significance of variety differences were calculated using a Tukey test and represented with letters. Correlations between traits were calculated on variety means in both years separately, as we were interested if correlations were similar in both years. SPSS (SPSS ver. 26, IBM) was used for all analysis.

3. Results

3.1. Weather Conditions

The data derived from the weather station of the German Meteorological Service (DWD) next to the experimental site in 2014 and 2015 are presented in Figure 1. The year 2014 showed favorable growing conditions in March, with higher temperatures and more radiation than in the following year (Figure 1). Although there was a higher global radiation in March 2015, air temperatures in April were similar for both years. Compared to some drought periods in April in both years, there was clearly more precipitation in May 2014 and 2015. In 2015, however, strong precipitation in May flooded some plots. Altogether, there was less precipitation in 2014 than in 2015. The grain filling phase in 2014 benefited from a high radiation budget in June. Although the plants were finally harvested in the end of July, the crops had already reached physiological maturity by mid-July in both years.

3.2. Grain Yield and Quality and Yield Components

ANOVA revealed that there was no significant difference among varieties in terms of grain yield, grain number per m^2, and grain number per spike, whereas significant differences occurred among the genotypes for grain protein content, grain retention fraction, HI, and yield components such as the mean grain weight (i.e., thousand grain weight (TGW)), and spike number per m^2, grain weight per spike and plant height (Table 2). Although differences between the two different years were generally significant for quality parameters and grain yield components of TGW, grain number per m^2, and per spike, there was no significant difference for grain yield alone, spike number per m^2, or grain weight per spike. There was no block effect for all parameters. Table 2 shows that there was no significant interaction of block x variety, and that the year × variety interaction was significant only for grain retention because of the difference in weather conditions between 2014 and 2015.

Table 2. Analysis of variance (ANOVA) (mean squares) of yield, quality parameters, yield components, and plant height of 23 spring malting barley varieties cultivated for 2 years at the same site under optimal N fertilization. Statistically significant differences are indicated as: *, $p < 0.05$; ***, $p < 0.001$; and NS, not significant.

Source of Variance	df	Grain Yield and Quality			Yield Components						Plant Height
		Yield	Protein Content	Grain Retention (>2.5 mm)	Grain Number m^{-2}	Spike Number m^{-2}	Grain Number $Spike^{-1}$	Grain Weight $Spike^{-1}$	TGW	HI	
Year (Y)	1	2.2 NS	201 ***	2153 ***	196,405,741 ***	32,309 NS	227 ***	0.01 NS	865 ***	0.004 NS	1.9 ***
Variety (V)	22	1.2 NS	2.5 ***	10 ***	6,530,199 NS	17,764 *	5.2 NS	0.03 ***	33 ***	0.010 ***	0.041 ***
Y × V	22	1.4 NS	0.43 NS	7.1 ***	7,762,290 NS	15,986 NS	3.3 NS	0.01 NS	3.5 NS	0.001 NS	0.006 NS
Block (B)	3	0.22 NS	0.26 NS	8.1 NS	1,666,474 NS	6078 NS	1.6 NS	0.004 NS	5.9 NS	0.002 NS	0.012 NS
B × V	66	0.72 NS	0.24 NS	1.9 NS	4,180,474 NS	7405 NS	3.7 NS	0.005 NS	5.0 NS	0.001 NS	0.006 NS

Grain yield in 2014 and 2015 was similar in both years (Tables 3 and 4). The mean grain yield from 23 genotypes was approximately 6.4 t ha^{-1} in 2014 and 6.6 t ha^{-1} in 2015. Although yield among the genotypes varied between 5.1 and 7.4 t ha^{-1} in 2014 and between 5.9 and 7.6 t ha^{-1} in 2015, there was no significant difference between the varieties in both years (Tables 3 and 4). Varieties with high yield were not consistent in both years; for example, the top five varieties showing high yield were Marthe, Salone, Solist, Union, and Wiebke in 2014, while they were Baronesse, Carina, Irona, Shakira, and Solist in 2015.

The malting and brewing industries require that the protein content ranges between 9.5% and 11.5% dry weight, and the grain retention fraction must be more than 90% of the harvested grain size >2.5 mm. In 2014, however, the protein content of more than 90% of the varieties was less than 9.5%, whereas in 2015, the grain retention fraction of grains >2.5 mm for about 40% of the varieties was less than 90% (Tables 3 and 4). Thus, the year significantly affected the malting quality properties. The mean protein content from 23 varieties was approximately 8.3% in 2014 and 10.4% in 2015, which was 25% higher than in 2015, while the mean grain retention fraction of grains >2.5 mm was approximately 97.5% in 2014 and 90.6% in 2015, which was 7% lower in 2015 than in 2014. In contrast to the grain yield, varieties with low protein content were not consistent over the two years, except for Irina, Solone, and Solist.

The results in Tables 3 and 4 show that the genotypic effects on yield components such as grain and spike number per m^2 in 2014 were different from those in 2015. For example, there was no difference in grain and spike number per m^2 in 2015, whereas a significant difference was found in 2014. The results show that there was no significant difference in grain number per spike among the barley genotypes. In contrast, a similar genotypic effect on grain number and weight per spike, TGW, and HI was observed in both years. There was a marked change in grain number per m^2 and per spike and TGW between the two years, while the spike number per m^2 moderately varied with years. For example, the grain number per m^2 was 15% higher in 2015 than in 2014; while the grain number per spike was 10% higher and the TGW was 9% less than that in 2014. However, there was no significant difference in grain weight per spike or HI between the years. The average plant height was greater in 2014 than in 2015.

Grain yield was significantly correlated with grain and spike number per m^2 in both years, while there was only a significant association between grain yield and grain number per spike in 2014 (Table 5). Although a negative relationship between protein content and grain yield was found in both years (Tables 5 and 6), this correlation was not significant. The protein content was negatively correlated with most of the yield components in 2014 and 2015. However, there were only significant correlations between protein content and parameters such as grain weight per spike, TGW, HI in 2014, and plant height in 2014 and 2015. In contrast, a significant relationship between protein content and TGW was only found in 2015.

Negative correlations between grain retention fraction and yield and yield components in 2014, except for TGW and HI, and plant height in 2014 and 2015 were observed, but the relationships were not significant (Table 5). In 2015, however, the grain retention fraction was positively correlated with yield and most of the yield components, except for the grain and spike number, whereas it was significantly correlated only with weight per spike, TGW, and HI. The grain number per m^2 was significantly associated with the spike number per m^2.

Table 3. Mean (±SE) and Tukey test for yield, quality properties, yield components, and plant height of 23 spring malting barley varieties in 2014. A mean comparison between varieties from Tukey´s HSD-test indicates a significant difference at $p < 0.05$. The same letters indicate groups in a column that were not significantly different from one another.

Variety Name	**Grain Yield and Quanlity**						**Yield Components**												**Plant Height**	
	Yield		**Protein Content**		**Grain Retention (>2.5 mm)**		**Grains**		**Spikes**		**Grains**		**Grain Weight**		**TGW**		**HI**			
	t ha^{-1}		**%**				**Number m^{-2}**				**Number Spike^{-1}**		**g Spike^{-1}**		**g**				**m**	
	Mean ± SE		**Mean ± SE**		**Mean ± SE**		**Mean ± SE**		**Mean ± SE**		**Mean ± SE**		**Mean ± SE**		**Mean ± SE**		**Mean ± SE**		**Mean ± SE**	
Aspen	5.7 ± 0.4	a	8.7 ± 0.4	a–d	97.7 ± 0.4	ab	12,614 ± 921	ab	643 ± 27.1	ab	19.6 ± 0.6	a	0.90 ± 0.04	ab	45.2 ± 0.14	a–c	0.55 ± 0.03	abc	0.80 ± 0.04	a–c
Barke	6.1 ± 0.7	a	8.4 ± 0.3	a–d	98.2 ± 0.1	b	13,197 ± 1562	ab	622 ± 61.6	ab	21.1 ± 0.6	a	0.98 ± 0.05	a–c	46.2 ± 0.62	a–c	0.60 ± 0.00	bc	0.73 ± 0.03	ab
Baronesse	5.8 ± 0.2	a	8.7 ± 0.3	a–d	97.2 ± 0.3	ab	12,430 ± 494	ab	592 ± 36.5	ab	22.0 ± 1.0	a	1.00 ± 0.04	a–c	45.7 ± 0.37	a–c	0.60 ± 0.00	bc	0.77 ± 0.04	a–c
Braemar	6.7 ± 0.5	a	7.9 ± 0.1	ab	98.2 ± 0.3	b	13,658 ± 917	ab	659 ± 30.4	ab	20.7 ± 0.8	a	1.00 ± 0.04	a–c	48.9 ± 0.87	bc	0.60 ± 0.00	bc	0.71 ± 0.03	ab
Carina	5.7 ± 0.5	a	8.4 ± 0.2	a–d	97.7 ± 0.2	ab	13,087 ± 992	ab	632 ± 47.9	ab	20.7 ± 0.4	a	0.93 ± 0.03	a–c	43.5 ± 0.48	ab	0.53 ± 0.03	ab	0.88 ± 0.05	cd
Grace	6.6 ± 0.5	a	8.5 ± 0.4	a–d	97.8 ± 0.6	ab	13,247 ± 746	ab	602 ± 53.9	ab	22.2 ± 0.8	a	1.10 ± 0.00	c	49.6 ± 0.97	c	0.60 ± 0.00	bc	0.65 ± 0.08	a
IPZ 24727	5.7 ± 0.7	a	9.6 ± 0.4	cd	97.1 ± 0.4	ab	11,838 ± 1454	ab	602 ± 101	ab	20.0 ± 0.8	a	0.98 ± 0.03	a–c	47.6 ± 0.56	bc	0.58 ± 0.03	abc	0.74 ± 0.08	a–c
Irina	6.9 ± 0.3	a	7.7 ± 0.2	ab	96.9 ± 0.3	ab	14,485 ± 766	ab	633 ± 20	ab	22.8 ± 0.6	a	1.10 ± 0.04	bc	48.0 ± 0.43	bc	0.60 ± 0.00	bc	0.67 ± 0.01	ab
Mackay	6.2 ± 0.5	a	8.0 ± 0.2	ab	97.6 ± 0.7	ab	12,778 ± 871	ab	583 ± 30.8	ab	21.9 ± 0.7	a	1.08 ± 0.03	c	48.8 ± 0.89	bc	0.60 ± 0.00	bc	0.77 ± 0.04	a–c
Marthe	7.4 ± 0.6	a	8.7 ± 0.4	a–d	97.6 ± 0.5	ab	16,612 ± 1310	b	793 ± 52.9	b	20.9 ± 0.4	a	0.95 ± 0.03	a–c	44.5 ± 0.42	a–c	0.60 ± 0.00	bc	0.70 ± 0.04	ab
Melius	6.6 ± 0.6	a	7.5 ± 0.2	ab	97.2 ± 0.5	ab	13,291 ± 938	ab	616 ± 49.9	ab	21.7 ± 0.7	a	1.08 ± 0.05	bc	49.5 ± 0.84	c	0.60 ± 0.00	bc	0.70 ± 0.02	ab
Power	7.0 ± 0.6	a	7.7 ± 0.2	ab	95.5 ± 0.4	a	14,945 ± 1228	ab	661 ± 54.1	ab	22.6 ± 0.3	a	1.05 ± 0.03	a–c	46.6 ± 0.44	a–c	0.60 ± 0.00	bc	0.66 ± 0.01	ab
Quench	6.7 ± 0.8	a	7.8 ± 0.3	ab	97.1 ± 0.5	ab	14,537 ± 1579	ab	665 ± 64.1	ab	21.8 ± 0.5	a	0.98 ± 0.03	a–c	45.8 ± 0.57	a–c	0.60 ± 0.00	bc	0.68 ± 0.03	ab
Salome	7.2 ± 0.6	a	7.3 ± 0.1	a	97.1 ± 0.4	ab	14,771 ± 1075	ab	713 ± 36.7	ab	20.7 ± 0.6	a	1.00 ± 0.04	a–c	48.4 ± 0.59	bc	0.63 ± 0.03	c	0.63 ± 0.04	a
Scarlett	6.4 ± 0.4	a	8.4 ± 0.4	a–d	97.8 ± 0.2	ab	14,538 ± 1004	ab	718 ± 80.6	ab	20.6 ± 1.0	a	0.93 ± 0.05	a–c	44.2 ± 0.78	a–c	0.58 ± 0.03	abc	0.64 ± 0.04	a
Shakira	5.1 ± 0.3	a	7.7 ± 0.2	ab	97.9 ± 0.4	ab	10,373 ± 507	a	492 ± 25.8	a	21.2 ± 0.9	a	1.05 ± 0.03	a–c	49.6 ± 0.41	c	0.60 ± 0.00	bc	0.73 ± 0.02	ab
Sissy	5.5 ± 0.1	a	8.7 ± 0.2	a–d	97.3 ± 0.2	ab	12,477 ± 220	ab	617 ± 19.1	ab	20.3 ± 0.6	a	0.90 ± 0.00	ab	43.2 ± 0.92	a	0.57 ± 0.03	abc	0.83 ± 0.07	a–c
Solist	7.4 ± 0.7	a	7.6 ± 0.4	ab	98.3 ± 0.5	b	16,057 ± 1380	b	704 ± 55.7	ab	22.8 ± 0.8	a	1.05 ± 0.03	a–c	46.1 ± 0.84	a–c	0.60 ± 0.00	bc	0.70 ± 0.04	ab
Trumpf	6.4 ± 0.5	a	8.1 ± 0.1	a–c	98.1 ± 0.5	b	13,873 ± 1151	ab	650 ± 44.6	ab	21.3 ± 0.4	a	1.00 ± 0.00	a–c	46.5 ± 0.84	a–c	0.60 ± 0.00	bc	0.75 ± 0.02	a–c
Union	7.0 ± 0.4	a	9.8 ± 0.4	d	96.9 ± 0.6	ab	15,306 ± 792	ab	686 ± 34.8	ab	22.4 ± 1.1	a	1.03 ± 0.05	a–c	45.9 ± 0.76	a–c	0.50 ± 0.00	a	0.96 ± 0.08	c
Ursa	6.1 ± 0.2	a	9.1 ± 0.5	b–d	97.3 ± 0.7	ab	13,387 ± 359	ab	633 ± 20.7	ab	21.2 ± 0.9	a	0.98 ± 0.05	a–c	45.8 ± 0.9	a–c	0.60 ± 0.00	bc	0.76 ± 0.02	a–c
Volla	6.1 ± 0.4	a	8.9 ± 0.4	a–d	97.4 ± 0.3	ab	14,139 ± 766	ab	710 ± 72.4	ab	20.3 ± 1.4	a	0.88 ± 0.05	a	43.2 ± 0.45	ab	0.50 ± 0.04	a	0.88 ± 0.02	cd
Wiebke	7.0 ± 0.3	a	8.5 ± 0.2	a–d	97.7 ± 0.1	ab	14,711 ± 653	ab	675 ± 35.4	ab	21.9 ± 0.2	a	1.03 ± 0.03	a	44.8 ± 0.5	a–c	0.60 ± 0.00	bc	0.78 ± 0.02	a–c
Mean	6.4		8.3		97.5		13,754		648		21.3		1.00		46.4		0.58		0.74	
Min	5.1		7.3		95.5		10,373		492		19.6		0.88		43.2		0.50		0.63	
Max	7.4		9.8		98.3		16,612		793		22.8		1.10		49.6		0.63		0.96	

Table 4. Mean (±SE) and Tukey test for yield, quality properties, yield components, and plant height for 23 spring malting barley varieties in 2015. A mean comparison between varieties from Tukey´s HSD–test indicates a significant difference at $p < 0.05$. The same letters indicate groups in a column that were not significantly different from one another.

Variety Name	Grain Yield and Quanlity			Yield Components							Plant Height
	Yield	Protein Content	Grain Retention (>2.5 mm)	Grains	Spikes	Grains	Grain Weight	TGW	HI		
	t ha^{-1}	%		Number m^{-2}		Number Spike^{-1}	g Spike^{-1}	g			m
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE		Mean ± SE
Aspen	6.2 ± 0.6 a	11.2 ± 0.0 ef	88.8 ± 0.7 a–d	15,452 ± 1,075 a	657 ± 49 a	23.6 ± 0.7 a	0.93 ± 0.05 ab	39.9 ± 0.9 a–c	0.58 ± 0.03 ab		0.58 ± 0.03 a–f
Barke	6.0 ± 0.5 a	10.3 ± 0.1 a–f	93.1 ± 0.8 cd	13,858 ± 981 a	561 ± 57 a	25.1 ± 1.3 a	1.08 ± 0.03 a–c	43.3 ± 1.3 b–e	0.60 ± 0.00 ab		0.53 ± 0.01 a–f
Baronesse	7.6 ± 0.9 a	10.2 ± 0.2 a–f	91.0 ± 0.4 a–d	18,078 ± 2,335 a	762 ± 76 a	23.7 ± 1.4 a	1.00 ± 0.04 a–c	42.0 ± 0.5 a–e	0.60 ± 0.00 ab		0.55 ± 0.03 a–f
Braemar	6.9 ± 0.3 a	10.7 ± 0.6 b–f	91.5 ± 0.7 a–d	16,334 ± 666 a	685 ± 37 a	23.9 ± 0.4 a	1.03 ± 0.03 a–c	42.5 ± 0.2 a–e	0.60 ± 0.00 ab		0.50 ± 0.01 a–c
Carina	7.4 ± 0.9 a	10.6 ± 0.2 a–f	88.1 ± 0.7 ab	18,632 ± 2,319 a	827 ± 88 a	22.5 ± 0.9 a	0.90 ± 0.04 ab	40.0 ± 0.5 a–c	0.53 ± 0.03 a		0.67 ± 0.02 f
Grace	7.0 ± 0.3 a	10.7 ± 0.2 b–f	93.7 ± 0.4 d	14,974 ± 468 a	587 ± 36 a	25.7 ± 0.8 a	1.18 ± 0.05 c	46.6 ± 0.7 e	0.63 ± 0.03 b		0.54 ± 0.02 a–f
IPZ 24727	6.9 ± 0.5 a	11.4 ± 0.4 f	90.9 ± 1.1 a–d	16,446 ± 897 a	699 ± 46 a	23.7 ± 1.1 a	0.98 ± 0.05 a–c	42.0 ± 0.7 a–e	0.58 ± 0.03 ab		0.64 ± 0.02 d–f
Irina	7.1 ± 0.4 a	9.5 ± 0.1 ab	88.8 ± 1.2 a–c	16,253 ± 751 a	707 ± 37 a	23.0 ± 0.2 a	1.00 ± 0.00 a–c	43.9 ± 0.7 c–e	0.60 ± 0.00 ab		0.44 ± 0.02 a
Mackay	6.2 ± 1.2 a	10.3 ± 0.1 a–f	89.0 ± 1.2 a–d	14,559 ± 2593 a	626 ± 90 a	22.9 ± 0.8 a	0.98 ± 0.05 a–c	42.6 ± 0.6 a–e	0.60 ± 0.00 ab		0.51 ± 0.05 a–e
Marthe	7.0 ± 0.2 a	10.4 ± 0.1 a–f	92.5 ± 0.6 b–d	16,823 ± 488 a	790 ± 20 a	21.3 ± 0.9 a	0.90 ± 0.04 ab	41.8 ± 0.4 a–d	0.60 ± 0.00 ab		0.54 ± 0.02 a–f
Melius	6.9 ± 0.4 a	10.0 ± 0.2 a–d	88.7 ± 1.3 a–c	15,411 ± 752 a	646 ± 25 a	23.8 ± 0.6 a	1.08 ± 0.03 a–c	44.6 ± 1.2 de	0.60 ± 0.00 ab		0.55 ± 0.02 a–f
Power	6.1 ± 0.4 a	10.0 ± 0.2 a–e	90.5 ± 1.3 a–d	14,197 ± 883 a	560 ± 46 a	25.8 ± 2.2 a	1.13 ± 0.10 bc	43.1 ± 0.6 b–e	0.60 ± 0.00 ab		0.50 ± 0.01 a–d
Quench	6.6 ± 0.7 a	10.0 ± 0.1 a–e	87.5 ± 1.3 a	16,556 ± 1826 a	715 ± 66 a	23.1 ± 1.3 a	0.90 ± 0.04 ab	39.9 ± 0.9 a–c	0.60 ± 0.00 ab		0.46 ± 0.02 a–f
Salome	5.9 ± 0.5 a	9.9 ± 0.2 a–c	93.2 ± 1.0 cd	13,393 ± 1048 a	627 ± 62 a	21.5 ± 0.5 a	0.93 ± 0.03 ab	44.2 ± 0.7 c–e	0.63 ± 0.03 b		0.43 ± 0.02 a
Scarlett	6.3 ± 0.8 a	10.7 ± 0.2 b–f	92.3 ± 0.8 a–d	15,367 ± 2130 a	611 ± 58 a	24.8 ± 1.5 a	1.00 ± 0.04 a–c	41.4 ± 0.6 a–d	0.60 ± 0.00 ab		0.45 ± 0.02 ab
Shakira	7.0 ± 0.9 a	10.3 ± 0.3 a–f	93.2 ± 0.5 d	16,043 ± 2278 a	724 ± 70 a	21.9 ± 1.0 a	0.95 ± 0.03 a–c	44.2 ± 0.6 c–e	0.60 ± 0.00 ab		0.49 ± 0.02 a–c
Sissy	6.3 ± 0.8 a	11.3 ± 0.2 ef	89.3 ± 1.3 a–d	16,084 ± 1513 a	690 ± 80 a	23.6 ± 1.2 a	0.90 ± 0.04 ab	39.0 ± 1.7 ab	0.58 ± 0.03 ab		0.63 ± 0.04 d–f
Solist	7.3 ± 0.5 a	9.3 ± 0.6 a	92.5 ± 0.1 b–d	17,029 ± 1483 a	725 ± 31 a	23.4 ± 1.3 a	1.03 ± 0.05 a–c	43.1 ± 0.9 b–e	0.60 ± 0.00 ab		0.54 ± 0.02 a–f
Trumpf	6.0 ± 0.2 a	10.5 ± 0.1 a–f	90.1 ± 0.7 a–d	14,664 ± 731 a	610 ± 39 a	24.2 ± 0.9 a	1.00 ± 0.04 a–c	41.0 ± 0.6 a–d	0.60 ± 0.00 ab		0.51 ± 0.01 a–e
Union	6.4 ± 0.5 a	10.7 ± 0.1 b–f	91.3 ± 0.8 a–d	15,502 ± 1617 a	662 ± 27 a	23.2 ± 1.6 a	0.95 ± 0.05 a–c	41.7 ± 1.1 a–d	0.60 ± 0.00 ab		0.62 ± 0.05 c–f
Ursa	6.6 ± 0.5 a	10.3 ± 0.2 a–f	88.8 ± 0.3 a–c	16,567 ± 1087 a	711 ± 26 a	23.3 ± 0.8 a	0.93 ± 0.05 ab	39.6 ± 0.7 a–c	0.58 ± 0.03 ab		0.56 ± 0.02 a–f
Volla	6.3 ± 0.2 a	11.1 ± 0.3 d–f	87.6 ± 0.5 ab	16,593 ± 574 a	710 ± 21 a	23.4 ± 0.6 a	0.88 ± 0.03 a	38.0 ± 0.6 a	0.55 ± 0.03 ab		0.64 ± 0.02 ef
Wiebke	6.3 ± 0.5 a	10.6 ± 0.2 b–f	92.0 ± 1.7 a–d	15,062 ± 1492 a	618 ± 55 a	24.4 ± 1.0 a	1.05 ± 0.03 a–c	42.0 ± 1.5 a–d	0.60 ± 0.00 ab		0.55 ± 0.03 a–f
Mean	6.6	10.4	90.6	15,821	674	23.5	0.98	42.0	0.59		0.54
Min	5.9	9.3	87.5	13,393	560	21.3	0.88	38.0	0.53		0.43
Max	7.6	11.4	93.7	18,632	827	25.8	1.18	46.6	0.63		0.67

Table 5. Pearson's correlation coefficient of grain yield, quality properties, yield components, and plant height of 23 spring malting barley varieties under optimal N fertilization in 2014. Statistically significant differences are indicated as: *, $p < 0.05$; **, $p < 0.01$; and ns, not significant.

	Grain Yield and Quality						Yield Components												Plant Height
	Yield		Protein Content		Grain Retention (>2.5 mm)		Grain Number per m^2		Spike Number per m^2		Grain Number per Spike		Grain Weight per Spike		TGW		HI		
Grain yield	1.00																		
Grain protein content	−0.31	ns	1.00																
Grain retention (>2.5 mm)	−0.16	ns	−0.02	ns	1.00														
Grain number per m^2	0.91	**	−0.14	ns	−0.15	ns	1.00												
Spike number per m^2	0.71	**	0.03	ns	−0.05	ns	0.90	**	1.00										
Grain number per spike	0.55	**	−0.31	ns	−0.29	ns	0.38	ns	−0.06	ns	1.00								
Grain weight per spike	0.39	ns	−0.42	*	−0.15	ns	0.04	ns	−0.35	ns	0.80	**	1.00						
TGW	0.15	ns	−0.44	*	0.01	ns	−0.23	ns	−0.43	*	0.35	ns	0.79	**	1.00				
HI	0.23	ns	−0.60	**	0.05	ns	−0.01	ns	−0.15	ns	0.26	ns	0.48	*	0.49	*	1.00		
Plant height	−0.18	ns	0.35	**	−0.09	ns	−0.09	ns	−0.01	ns	−0.16	ns	−0.28	**	−0.25	*	−0.47	**	1.00

Table 6. Pearson's correlation coefficient of grain yield, quality properties, yield components, and plant height of 23 spring malting barley varieties under optimal N fertilization in 2015. Statistically significant differences are indicated as: *, $p < 0.05$; **, $p < 0.01$; and ns, not significant.

	Grain Yield and Quality						Yield Components												Plant Height
	Yield		Protein Content		Grain Retention (>2.5 mm)		Grain Number per m^2		Spike Number per m^2		Grain Number per Spike		Grain Weight per Spike		TGW		HI		
Grain yield	1.00																		
Grain protein content	−0.22	ns	1.00																
Grain retention (>2.5 mm)	0.01	ns	−0.11	ns	1.00														
Grain number per m^2	0.81	**	0.07	ns	−0.36	ns	1.00												
Spike number per m^2	0.73	**	−0.01	ns	−0.31	ns	0.91	**	1.00										
Grain number per spike	−0.24	ns	0.16	ns	0.12	ns	−0.34	ns	−0.69	**	1.00								
Grain weight per spike	0.01	ns	−0.25	ns	0.45	*	−0.43	*	−0.65	**	0.77	**	1.00						
TGW	0.20	ns	−0.48	*	0.63	**	−0.41	ns	−0.39	ns	0.18	ns	0.74	**	1.00				
HI	−0.17	ns	−0.41	ns	0.64	**	−0.61	**	−0.55	**	0.17	ns	0.53	**	0.73	**	1.00		
Plant height	0.59	**	0.44	**	−0.12	ns	0.53	**	0.51	**	0.09	ns	−0.02	ns	−0.24	*	−0.35	**	1.00

4. Discussion

The average grain yield of spring malting barley from 23 genotypes in the current study was about 6.4 t ha^{-1} in 2014 and 6.6 t ha^{-1} in 2015, respectively (Tables 3 and 4), which was slightly higher than the average yield of spring barley in the same years across different regions in southern Germany. The Bavarian State Research Center for Agriculture has conducted several long-term experiments in different regions in southern Germany, and accordingly reported that the grain yield of spring barley in 2014 and 2015 reached more than 6 t ha^{-1} across the Bavarian State, which was the highest level recorded, compared to the average yield of about 4.8 t ha^{-1} between 2004 and 2014 [43,44].

Our study showed a strong correlation between grain yield and grain number m^{-2} in both years (Tables 5 and 6), supporting previous findings in the literature across a range of environments [10,13,16,18,19,45,46]. Furthermore, in this study, grain yield was not significantly associated with grain weight (Tables 5 and 6). Gallagher et al. [13] and Bulman et al. [20] reported a similar finding that the mean grain weight tended to be less variable and was poorly correlated with yield.

Grain number per m^2 is the product of the number of spikes that remain at crop maturity with grain-bearing spikes that depend on tillering at early growth stages and tiller abortion in the later growth stages, and the number of grains per spike. An increase in grains per m^2 of wheat varieties from breeding progress in past decades was primarily attributed to increases in spikes per m^2 with little variation in grains $spike^{-1}$ observed, while similar studies in warmer regions such as Argentina have reported significant variation in grains $spike^{-1}$ among genotypes [25]. The results from the present study demonstrate that there was a strong relationship between grain number m^{-2} and spikes m^{-2}, while a poor correlation was found between grains m^{-2} and grain $spike^{-1}$ (Tables 5 and 6), which was more similar to the behavior of wheat genotypic variation in a West European climate. Therefore, this finding suggests that, to further increase the grain yield of spring barley under weather conditions in southern Germany, a high spike number per m^2 should be achieved during the vegetative growth stages.

The number of spikes at harvest depends on many developmental factors, including plant establishment, tillering dynamics, and tiller abortion to anthesis. In 2014, for example, the weather was favorable for spring barley. After a very dry and mild winter (the third warmest since weather records began in 1881 [43]), the plants began to grow very early that year. Similarly, in 2015, the warm and dry weather in March caused the soil to dry quickly, which enabled sowing of spring barley in good time and under good conditions. However, the grain number per m^2 was higher in 2015 than in 2014. This was due to a higher spike number per m^2 and more grains per spike in 2015. The lower number of spikes per m^2 in 2014 may have been due to drought periods in April, May, and June (Figure 1). In April, for example, the precipitation was approximately 30 mm in 2014 but was about 60 mm in 2015. A dry period also occurred in the middle of May, 2014. Drought periods during vegetative growth stages may have inhibited tillering in 2014 compared to 2015. Although the 23 spring barley genotypes were registered in different time periods and regions (Table 1), this study surprisingly showed no significant genotypic variation in grain yield (Tables 3 and 4).

Grain protein content is one of the most important factors in marketing malting barley. The primary objective is to maintain grain protein content between 9.5% and 11.5%. Studies in the literature have shown that grain N in cereals mainly represents N supply and N uptake in the vegetative organs until anthesis and the translocation of N reserves to grains during the grain filling phase [22,47,48]. Studies have revealed that approximately 90% of N reserves translocates to grains during the grain filling phase [47,48]. Compared to the contribution of N translocation to grain N at the final harvest, the N uptake before anthesis may play a more important role in wheat [49], whereas Bulman and Smith [49] found genotypic variation in N uptake and translocation to the grain during the process of grain filling in barley. In this study, the N fertilizer application rate for spring malting barley was based on the official recommendation for southern Germany. Although the N supply rate was even lower in 2015, 22 barley varieties among 23 obtained protein content between 9.5% and 11.5% in 2015. The protein content varied with genotypes, ranging from 9.3% to 11.4%. In contrast, the protein content of only two varieties, IPZ 2427 and Union, reached more than 9.5% in 2014, with the protein content of the 23 genotypes ranging from 8.3% to 9.8%. Since IPZ 2427 is a new variety that has not yet been registered, while Union was registered in 1950 (Table 1), our results suggest that the protein content from all modern spring barley varieties in 2014 could not meet the quality requirements for the brewing industry.

Owing to the higher N supply compared to that in 2015, the low protein content in 2014 seemed primarily to be associated with the N uptake before anthesis. Our results revealed that N uptake before anthesis was about 82 kg N ha^{-1} in 2014 and 99 kg N ha^{-1} in 2015, meaning the N uptake was 20% lower in 2014 than in 2015. Drought is a major factor that inhibits both N uptake and translocation. Compared to the weather in 2015, drought spells appeared more often in 2014, especially in April and May, the period before anthesis (Figure 1). After anthesis, a further drought period occurred in June 2014 (Figure 1), indicating that a reduction in N translocation in 2014 could also not be excluded.

Overall, this study suggests that in order to ensure that the protein content meets the quality requirement, the N application rate at the same site should vary with year when weather is different between years, suggesting that the environmental effects had more influence on total variation than genotypic effects. Currently, sensing technology is available for in-season N fertilisation of field crops. Differences in N status can already be detected in early and late tillering stages [50]. Barmeier et al. [51] and Barmeier and Schmidhalter [52] reported that spectral sensing techniques can be used to recommend a more targeted N application for spring barley, since this not only allows for the detection of actual growth and N status, but can also be used to estimate soil nitrogen mineralisation [53], which could be used for a targeted second N application correcting for possible nitrogen deficiency and avoiding surplus nitrogen fertilisation.

Although grain size contributed less to grain yield in both years, grain size is one of the most important parameters for malting barley. To meet the quality requirements of the brewing industry, the proportion of the grain with size > 2.5 mm must be greater than 90%. In contrast to no genotypic variation in grain yield, a significant effect of genotype and year was clearly observed on grain size (Table 2), which is in agreement with the results of a previous study on the effects of genotypic and environmental factors on grain size [5]. More interestingly, however, all barley varieties in 2014 showed more than 90% of grains that were larger than 2.5 mm, while only about 60% of the varieties in 2015 showed 90% grains that were greater than 2.5 mm. Since the grain weight and size are primarily determined during the post-anthesis period [16,17,23,24], this is likely due to the inhibition of grain filling during post-anthesis. Water and heat stresses and low radiation during the grain-filling period have a negative effect on barley grain weight and size. Drought and heat stress mainly induce a reduction in grain filling duration [20,30–33], whereas low radiation likely leads to a reduction in the grain filling rate [16,17,19,26,31,34], as up to 90% of the grain dry matter of barley is acquired by photosynthesis during grain filling [54,55]. From the beginning of anthesis to dough ripening in 2015, the radiation intensity was 25% lower compared to the same period in 2014. In addition, the higher spike number per m^2 in 2015 may result in higher shade between plants. Kennedy et al. [26] reported that the shade between plants could cause a reduction in grain size during grain filling. In 2015, the plants received more rainfall in June than in 2014. These results may indicate that radiation may play an important role in grain size during grain filling of spring barley. From the long-term experiment, the study by the LfL concluded that barley usually shows higher yield and better quality under sunny and drier conditions than in constantly cool and humid weather with a good water supply [43,44]. Furthermore, although there was a lack of evidence to show that drought and heat stress impacted grain filling in 2015, the grain filling duration was shortened because anthesis in 2015 began one week later than in 2014.

5. Conclusions

The results of this study show that there was little genotypic variation in grain yield over the two years, whereas variation in grain retention fraction was related to an interactive effect of year and genotype. In contrast, grain protein content was more affected by the year. Since grain yield was closely related to spikes per m^2, our study highlights the importance of tiller formation and establishment as decisive factors influencing malting barley yield. Regarding weather effects, the global radiation intensity during the post-anthesis phase was the major factor affecting final grain size. Grain protein content was primarily dependent on the year effect, suggesting that optimal N fertilisation levels must be varied between years in order to ensure the protein content meets the needs of the brewing industry. Since the same cultivars can have large variations in yield and protein concentration depending on the year, we strongly recommend further development of strategies addressing soil N mineralisation and N fertilisation to optimise the production of spring malting barley. With optimal management strategies, it seems that the varieties IPZ 24727, Marthe, and Salome would better achieve the requirements for both grain protein content and grain size simultaneously compared to other varieties.

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