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ORIGINAL PAPER

Environmental control of malting barley response to nitrogen in the Pampas, Argentina

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Abstract

Twenty-five single-year field experiments were established in order to assess the effects of nitrogen fertilization on grain yield, size, and protein concentration, and to explain its response to fertilization with soil, climate, and crop management variables easy to collect. While grain yield in control treatments was positively related to rainfall during the full crop cycle and negatively related to temperature during the critical period previous to heading, grain yield response to nitrogen was positively related with the product of fertilizer nitrogen rate by rainfall. Grain protein concentration response to nitrogen fertilization was positively related to fertilizer nitrogen rate and negatively related soil nitrate. It is worth noting that the effect of N fertilization on grain protein concentration was not conditioned by rainfall. We could establish that grain protein concentration was determined by the ratio between nitrogen availability (soil nitrogen-nitrate at sowing plus nitrogen added as fertilizer) and grain yield.

Keywords Barley · Nitrogen · Crop quality · Nutrient management · Grain size · Environmental control

Abbreviations

GYN_0	Grain yield in the control treatment
GYR	Grain yield response to N fertilization
Navail/GY	N availability by grain yield

Introduction

About 15% of world barley (*Hordeum vulgare* L.) production is converted into malt, which is the primary input for the manufacture of beer (Blake et al. 2011). To meet maltsters' quality requirements, barley grain must have a specific protein level, and high grain size (i.e. a high proportion of plump grains) (Briggs 1998). As the price of barley grains apt for malting is usually higher than those that do not meet these quality requirements, farmers try to achieve high yields and malting quality simultaneously. Grain yield, protein concentration, and size may be affected by nutrient deficiencies, drought or heat stress during the crop cycle (Fathi et al. 1997; Passarella et al. 2008; O´Donovan et al. 2011, 2015).

Barley is a crop of growing importance in the Pampas, the main region for grain crops in Argentina (Lavado and Taboada 2009). As in other agricultural regions of the world, nitrogen (N) is the nutrient that most frequently affects crop production (Stewart et al. 2005; Lavado and Taboada 2009). As a consequence of nutrient deficiency, fertilizer use has increased substantially during the last two decades (Lavado and Taboada 2009). A common effect of N fertilization on malting barley crops is to increase grain protein and to decrease grain size (O´Donovan et al. 2011, 2015; Sainju et al. 2013).

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Nitrogen applied in excess of crop needs may have detrimental effects on both farmer economy and the environment (Black 1993; Vitousek et al. 1997). Knowing the crop yield response to N rate is a basic tool for designing a sound fertilizer program. In malting barley, it is also relevant to know how grain protein content and size change with N availability. The simplest yield response functions for barley only include fertilizer rate as independent variable (e.g. O´Donovan et al. 2011; Sainju et al. 2013). However, more common response functions also include soil N availability at sowing. In the Pampas and other agricultural regions, yield response functions for wheat and other grain crops include the amount of N as nitrate in the top 60 cm of soil (Grant et al. 1991; Barbieri et al. 2009; Bell et al. 2013; Reussi Calvo et al. 2013). These models that only include soil availability at sowing are easy to use, but do not take into account N mineralization from organic matter that could be an important source of N for a crop (Campbell et al. 2008). In some cases, the inclusion of variables that allow for organic N mineralization provided more reliable predictions of crop yield response to N fertilization (Reussi Calvo et al. 2013). In addition, yield response to N application depends on crop requirement which is associated with the yield potential for a given environment (Bell et al. 2013). Therefore, any climatic, soil, and crop system factor that affect crop yield or organic matter mineralization may affect crop response to N application. The inclusion of the amount of rainfall, previous to or during the crop cycle in a model increased the explanation of yield variation of wheat in the Pampas and barley in the Canadian Great Plains (Bole and Pittman 1980; Reussi Calvo et al. 2013).

Relationships of N availability with grain protein concentration or size are usually more difficult to explain than with grain yield. In some cases, grain protein and size variation could be explained by just the rate of N applied or the amount of soil nitrate at sowing plus applied N (e.g. O´Donovan et al. 2011; Sainju et al. 2013). As grain protein concentration may be affected by pre or post anthesis drought, some models that explain its variation include variables that account for soil water availability. Dalal et al. (1997) observed that changes in grain protein concentration of wheat and barley in subtropical environments in Australia were related to the ratio of soil water content till 120 cm deep at sowing and N availability (i.e. soil nitrate till 120 cm deep at sowing plus applied N).

The objectives of this work were to explain grain yield, size, and protein responses to N fertilization in barley crops in the Pampas with soil, climate, and crop management variables easy to collect.

Materials and Methods

Twenty-five single-year experiments were established in the Pampas, Argentina, during 4 years (2005–2008). Fields were selected to represent the two main zones of barley production in Argentina (north and south of Buenos Aires province). At every experiment the effects of N fertilization were evaluated. Table 1 shows summarized information about the location, management practices, and soil for each experimental site.

The experiments were established at farmers' fields, and management practices were those normally used at each farm. In experiments established in the north of Buenos Aires province (sites 11–25) previous crop was always soybean, while in the south previous crops were several different grain crops. Fourteen experiments were under no-till management while the rest were conventional tilled. Barley cultivar was Scarlett at every site. Sowing date ranged from June 19 to July 20. To avoid phosphorus deficiency, 20 kg ha^{-1} of phosphorus was applied as triple superphosphate at every experimental site.

Randomized complete block designs with three replications were used for all experiments except at sites 14–19 where four replications were used. Plots were 10–20 m in length and 2–4 m in width across sites, as determined by the space available and the planter width.

The treatments were four rates of N fertilization (except at sites 4, 6, 8, and 10 where only three rates were used) (Table 1). Following a common practice in Argentina, N rates were established in order to reach certain amount of the sum of N as nitrate in the top 60 cm of soil plus the applied N. Therefore, the N rates varied between experiments. Nitrogen was applied as broadcast urea, at sowing in the experiments of the north of Buenos Aires (sites 11–25) and at tillering in the experiments of the south of Buenos Aires (sites 1–10) following management practices usually applied in each zone.

Rainfall, temperature and radiation data were obtained from the nearest weather stations. The amount of rainfall from June to November was from 154 to 447 mm, suggesting that a wide range of water availability was explored (Table 1). In order to analyze the relationships between yield, protein and grain size and climatic information, rainfall was summed and temperature and radiation were averaged for different time periods: (1) during the full cycle (between sowing and physiological maturity), (2) during the critical period when the number of grains is determined (from 40 to 10 days before heading) (Arisnabarreta and Miralles 2008), and (3) during the grain filling period (from heading to physiological maturity).

At each site, soil samples were collected before sowing from depths of 0 to 20, 20 to 40, and 40 to 60 cm. Carbon

Table 1 Selected site information and N rates used for 25 field trials

Site	Year	Location	Till	Crop-ping[a] (years)	Previous crop	Rainfall[b] (mm)	Soil type	pH 0–20 cm	OM[c] 0–20 cm (%)	Nitrate–N 0–60 cm (kg ha^{-1})	N rates			
											N$_0$ (kg ha^{-1})	N$_1$ (kg ha^{-1})	N$_2$ (kg ha^{-1})	N$_3$ (kg ha^{-1})
1	2005	S.F. Belloq	DH	> 20	Sunflower	280	T. Hapludolls	6.5	4.5	28	0	30	60	90
2	2006	S.F. Belloq	DH	> 20	Soybean	294	T. Hapludolls	6.2	4.9	37	0	24	54	84
3	2005	M.Cascallares	NT	> 10	Wheat	257	T. Argiudolls	6.5	2.3	39	0	42	72	102
4	2006	M.Cascallares	NT	> 10	Wheat	328	T. Hapludolls	6.4	3.1	55	0	12	42	72
5	2005	Puan	DH	6	Oats	211	T. Haplustoll	6.8	2.8	60	0	30	60	90
6	2006	Puan	DH	5	Sunflower	154	T. Haplustoll	6.5	2.3	60	0	31	61	91
7	2005	C. Suarez	DH	>10	Wheat	211	T. Argiudolls	6.2	4.1	70	0	38	68	98
8	2006	C. Suarez	NT	12	Wheat	297	T. Argiudolls	6.3	5.1	90	0	48	78	108
9	2007	C. Suarez	DH	10	Wheat	273	T. Argiudolls	6.0	3.3	48	0	29	59	–
10	2007	C. Suarez	DH	10	Wheat	273	T. Argiudolls	6.5	4.1	24	0	8	38	68
11	2005	Dennehy	DH	7	Soybean	382	E. Hapludolls	6.0	2.2	14	0	36	68	98
12	2006	Anderson	NT	5	Soybean	435	E. Hapludolls	6.1	2.7	62	0	17	47	77
13	2006	Anderson	NT	5	Soybean	435	E. Hapludolls	6.1	2.7	62	0	12	42	72
14	2005	Baigorrita	DH	> 10	Soybean	274	T. Hapludolls	5.8	2.4	71	0	30	60	–
15	2006	Baigorrita	DH	> 10	Soybean	423	T. Hapludolls	5.8	2.4	45	0	21	51	81
16	2008	Baigorrita	DH	> 10	Soybean	339	T. Hapludolls	5.4	2.5	42	0	9	39	69
17	2005	Junín	NT	> 20	Soybean	335	E. Hapludolls	5.5	1.3	46	0	30	60	–
18	2006	Junín	NT	> 10	Soybean	422	E. Hapludolls	5.4	2.4	49	0	24	54	–
19	2008	Junín	NT	> 20	Soybean	350	E. Hapludolls	5.2	2.1	34	0	20	50	80
20	2005	Arribeños	NT	> 20	Soybean	324	T. Hapludolls	5.5	3.6	62	0	40	60	80
21	2006	Arribeños	NT	> 10	Soybean	447	T. Hapludolls	5.6	2.8	80	0	60	80	100
22	2007	Arribeños	NT	> 10	Soybean	215	T. Hapludolls	5.4	2.0	61	0	9	39	69
23	2008	Arribeños	NT	> 10	Soybean	307	T. Hapludolls	6.1	2.7	39	0	30	60	90
24	2005	La Trinidad	NT	> 20	Soybean	297	T. Argiudolls	5.8	2.4	88	0	25	55	85
25	2006	La Trinidad	NT	> 10	Soybean	336	T. Argiudolls	5.6	2.7	61	0	21	51	81

T Typic, *E* Entic, *DH* disk harrow tillage, *NT* no-till

[a] Organic mater

[b] Rainfall during the full crop cycle

[c] Years of continuous cropping from the last pasture

content, and pH was determined at 0 to 20 cm depth, while N-nitrate was determined at 0 to 60 cm depth. Carbon content was converted to organic matter using a factor of 0.58 (Table 1).

At crop maturity, each plot was harvested and threshed by hand. Grain samples were oven dried at 65 °C to determine grain yield, size, and protein concentration. Grain yield was adjusted to a standard moisture content of 12%. Grain size was determined by size fractionation with a screening machine (Sortimat) with three slotted sieves of different widths (2.8, 2.5, and 2.2 mm). Each grain sample was separated into four grain size fractions: >2.8 mm (fraction 1), 2.8–2.5 mm (fraction 2), 2.5–2.2 mm (fraction 3), and <2.2 mm (fraction 4). Grain size (percentage >2.5 mm) was calculated as the percentage by weight of plump grains (fractions 1 plus 2) within each sample. Nitrogen concentration was determined by near-infrared spectroscopy (Foss 6500), calibrated with the Kjeldahl method. Protein concentration was calculated by multiplying the N concentration by a factor of 5.8. At 15 experimental sites, grain number and individual weight were also determined. Average individual grain weight was determined by counting and weighing three 100-grain subsamples taken from each plot at harvest. Grain number per unit area was calculated by dividing grain yield by individual grain weight. Yield, protein and size response to N fertilization was calculated as the difference between each N fertilized treatment (N_1, N_2 or N_3) and N_0 in each experimental site.

Treatment effects (N rate) were estimated with a mixed linear model using lme4 package in R (Bates et al. 2015; R Core Team 2016). The best linear unbiased estimators (BLUEs) were estimated by restricted maximum likelihood (REML). Analysis of variance and hypothesis tests were performed using lmerTest and lsmeans packages. N rate was considered as fixed effects. Location was treated as fixed because of need to estimate Location effect (i.e. average N rate in each specific Location). Location × year combinations (25 environments) and blocks within environments were considered as random effects. Multiple regression analysis was used in order to estimate grain yield, protein, and size in control plots and yield, protein and size response to N fertilization as a function of management, soil, and weather variables. Collinearity among independent variables was assessed by using the variance inflation factors. Variables that were significant at 0.05 level and had variance inflation factor values less than 2 were left in the final model.

Results

Grain Yield

The wide geographic distribution of the experimental sites and the variation of weather conditions resulted in a large variation of grain yield: from 1.5 to almost 7 t ha^{-1}. Significant responses to the N fertilization in 9 of 25 trials were observed (Tables 2 and 3). At these responsive sites, grain yield increased from 24 to 45%, which represented increases of more than one tonne in six trials. Environments with low yield potential (assessed by the maximum yield achieved at each site) showed no response to N fertilization. The largest responses to N fertilization were always observed in high yielding environments, even though some low responses were also observed in these environments.

Grain yield in the control treatment (GYN_0) and grain yield response to N fertilization (GYR) were not related to soil nitrate content at sowing. GYR was very weakly related to N rate ($p=0.05$; $R^2=0.054$). When other environmental variables were taken into account (pH and organic matter in the topsoil, rainfall, etc.), it was established that GYN_0 was significantly and positively related with rainfall during the full crop cycle and negatively related to mean temperature during the critical period when the number of grains is determined (Table 4). On the other hand, GYR was negatively related with soil organic mater and mean temperature during the full crop cycle, and positively related with the product of N rate by rainfall (full cycle). GYR was 480 kg ha^{-1} higher in Argiudolls than in other soils.

Grain Yield Components

Grain number per unit area varied much more than individual grain weight. The highest value of grain number more than quadrupled the lowest value, while in the case of individual grain weight the highest value was 1.38 times the lowest. Of the 15 experimental sites where yield components were measured, the N fertilization significantly increased grain number at nine sites (Tables 2 and 5).

Table 2 P values from the analysis of variance for the effects of N rate and environment (location × year), and their interaction on malting barley variables

Effects	Grain yield	Grain number	Individual grain weight	Grain size	Grain protein concentration
N rate (N)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Environment (E)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
N × E	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Significant effects ($p<0.05$) are in bold

Table 3 Malting barley grain yield, grain size and grain protein content as affected by nitrogen (N) fertilization at each experimental site

Site	Grain yield					Grain size					Grain protein concentration				
	N_0 (t ha^{-1})	N_1 (t ha^{-1})	N_2 (t ha^{-1})	N_3 (t ha^{-1})	*p* value	N_0 (%)	N_1 (%)	N_2 (%)	N_3 (%)	*p* value	N_0 (mg g^{-1})	N_1 (mg g^{-1})	N_2 (mg g^{-1})	N_3 (mg g^{-1})	*p* value
1	2.711	3.182	3.205	2.868	0.624	99.0	98.2	96.8	95.8	0.330	93	106	119	124	**< 0.001**
2	5.285	5.409	4.812	5.047	0.293	82.0	80.1	68.3	65.5	**< 0.001**	84	96	109	120	**< 0.001**
3	5.272	6.433	6.720	6.916	**< 0.001**	96.0	97.0	95.6	93.7	0.454	70	73	84	91	**< 0.001**
4	4.126	4.806	3.971	–	0.603	86.0	83.0	70.7	–	**< 0.001**	95	110	121	–	**< 0.001**
5	1.614	1.785	1.480	1.868	0.725	64.7	62.9	55.3	65.7	0.691	116	124	145	138	**< 0.001**
6	1.818	1.951	2.171	–	0.389	80.7	79.3	82.4	–	0.637	106	130	145	–	**< 0.001**
7	4.166	5.240	5.412	5.971	**< 0.001**	97.8	97.9	98.4	98.5	0.823	76	84	88	97	**< 0.001**
8	3.808	4.676	5.086	–	**0.002**	95.1	91.8	88.9	–	0.083	68	74	83	–	**0.016**
9	3.048	3.715	3.768	3.841	**0.041**	94.1	93.2	91.6	90.8	0.367	71	80	84	88	**0.011**
10	3.710	4.558	4.611	4.787	**0.005**	91.6	76.4	77.7	84.6	**0.003**	75	97	102	104	**< 0.001**
11	5.102	6.241	6.255	6.628	**< 0.001**	97.0	95.8	92.5	92.1	0.108	81	92	103	111	**< 0.001**
12	5.109	5.374	5.903	5.643	0.135	94.6	95.6	93.3	92.2	0.344	85	90	95	97	**0.049**
13	5.157	5.937	5.714	5.943	0.091	94.8	94.8	95.8	94.9	0.886	85	92	95	99	**0.018**
14	6.041	5.676	6.703	–	0.061	91.6	91.1	87.5	–	0.179	57	65	74	–	**0.002**
15	5.093	4.888	5.315	5.334	0.266	88.8	87.9	82.4	75.4	**< 0.001**	82	85	84	82	0.926
16	5.384	5.381	5.340	5.680	0.428	88.2	89.3	90.0	89.1	0.745	71	74	88	88	**< 0.001**
17	2.857	3.519	3.294	3.566	0.104	88.9	87.2	82.2	76.6	**< 0.001**	73	71	89	97	**< 0.001**
18	3.479	4.027	3.379	4.360	0.081	95.0	93.8	90.7	89.8	**0.049**	73	81	84	90	**0.003**
19	1.636	1.690	1.764	1.915	0.412	71.7	67.8	65.1	65.3	**0.028**	82	84	100	93	**0.002**
20	5.917	5.316	5.999	5.203	0.247	92.3	93.8	93.6	90.6	0.667	89	84	84	92	0.617
21	2.876	3.343	4.183	3.690	**0.015**	91.4	85.9	87.4	91.5	0.748	110	114	110	114	0.617
22	2.497	2.751	2.979	2.839	0.405	–	–	–	–	–	95	97	105	111	**0.003**
23	1.209	1.621	1.509	1.473	0.597	57.2	57.4	54.7	50.5	**0.042**	129	147	148	154	**< 0.001**
24	3.958	4.374	4.783	4.910	**0.017**	88.7	87.7	90.4	90.0	0.524	93	85	89	90	0.963
25	3.147	3.321	3.645	4.108	**0.012**	97.2	96.8	95.9	94.1	0.343	97	98	106	109	**0.034**

p values in bold denotes significance ($p < 0.05$)

Table 4 Models and model parameters for different soil and climates variables

Dependent variable	Model	R^2	Adjusted R^2	RSME	*p*
Grain yield in control treatments (t ha^{-1})	$y = 11.278 + 0.00908\ R_{FC} - 0.57879\ T_{CP}$	0.289	0.224	1596696	< 0.02
Grain yield response to N (t ha^{-1})	$y = 3.35126 + 0.48043$ ARG -0.2093 OM -0.1791 T_{FC}+ 0.00001418 NR x R_{FC}	0,392	0,355	177541	< 0.01
Grain size in control treatments (%)	$y = -\ 0.07255\ T^2_{GF}$	0.441	0.415	65.499	< 0.01
Grain protein concentration response to N (mg g^{-1})	$y = -76.1728 + 0.1610$ NR - 0.1589 SN + 2.658 LAT	0.581	0.562	0.53095	< 0.01

R_{FC} is the rainfall (mm) during the full crop cycle; T_{CP} is mean temperature (°C) during the critical period for grain number determination (40 days previous to heading); ARG is 1 when the soil is an Argiudoll and 0 when not; OM is organic mater content in the top 20 cm of the soil (%); T_{FC} is the mean temperature (°C) during the full crop cycle; NR is nitrogen fertilization rate (kgN ha^{-1}); T_{GF} is mean temperature (°C) during the grain filling period; SN is nitrate–N in the top 60 cm of the soil (kg N ha^{-1}); LAT is latitude (°)

Table 5 Grain number per unit area and individual grain weight as affected by nitrogen (N) fertilization

Site	Grain number per unit area					Individual grain weight				
	N_0 (m^{-2})	N_1 (m^{-2})	N_2 (m^{-2})	N_3 (m^{-2})	*p* value	N_0 (mg)	N_1 (mg)	N_2 (mg)	N_3 (mg)	*p* value
5	4690	5252	4356	5453	0.682	34.4	34.0	34.1	34.3	0.921
6	5362	5818	6468		0.304	37.9	37.7	37.6		0.797
7	10108	12441	13103	14284	**< 0.001**	42.1	42.1	41.3	41.8	0.664
8	10102	13074	15062		**< 0.001**	42.3	40.1	37.8		**< 0.001**
11	11645	15651	16405	17118	**< 0.001**	43.3	40.1	38.1	38.7	**< 0.001**
12	12249	12657	15266	14037	**0.027**	41.7	42.7	38.7	40.3	**0.023**
13	12484	14395	13700	14402	0.127	41.3	41.3	41.7	41.3	0.922
14	16484	15849	19235		**0.003**	36.8	35.8	34.9		0.072
15	12784	12384	14052	14435	**0.020**	40.0	39.6	37.9	37.0	**0.001**
16	13794	13822	13513	14565	0.4730	39.2	39.0	39.4	39.0	0.996
17	7762	9918	9319	10384	**0.018**	36.9	35.5	35.6	34.4	**0.025**
18	8603	10051	8495	11170	**0.040**	40.5	40.1	39.8	39.1	0.158
19	5068	5354	5477	5863	0.395	32.3	31.5	31.7	32.6	0.661
20	14670	13910	15364	13955	0.828	40.3	38.3	39.0	37.3	**0.022**
24	10325	11028	12469	13147	**0.004**	38.3	39.7	38.3	37.3	0.148

p values in bold denotes significance ($p < 0.05$)

Individual grain weight varied between 31 and 43 mg. Nitrogen fertilization significantly decreased individual grain weight at six sites (Tables 2 and 5). Considering all treatments and sites where yield components were evaluated, grain yield was positively and significantly associated with both grain number and individual grain weight, although the association with grain number was much closer than with individual grain weight (Eqs. 1 and 2). The association with individual grain weight was curvilinear, indicating that the variation of individual grain weight was much less with high grain yields than with low yields.

$$GY\ = 4.05910^{-4}GN - 0.255\left(R^2 = 0.96,\ p < 0.01,\ n = 57\right) \tag{1}$$

$$GY\ = -0.04081\ IGW^2 + 3.3859\ IGW - 65.045\left(R^2 = 0.45;\ p < 0.01;\ n = 57\right) \tag{2}$$

where GY is grain yield (t ha^{-1}), GN is grain number (grains m^{-2}), and IGW is individual grain weight (mg).

Grain Size and Size Fractions

Grain size (percentage > 2.5 mm) varied widely among sites and treatments, with values from 50 to 99% (Table 3). N fertilization significantly decreased grain size at 10 sites. Grain size in control treatments was negatively related to the mean temperature during the grain filling period (Table 4). Grain size response to N fertilization was not related to any environmental variable measured in this study.

Grain size was positively associated with individual grain weight (Fig. 1a). This relationship was curvilinear indicating that, when the individual grain weight was relatively

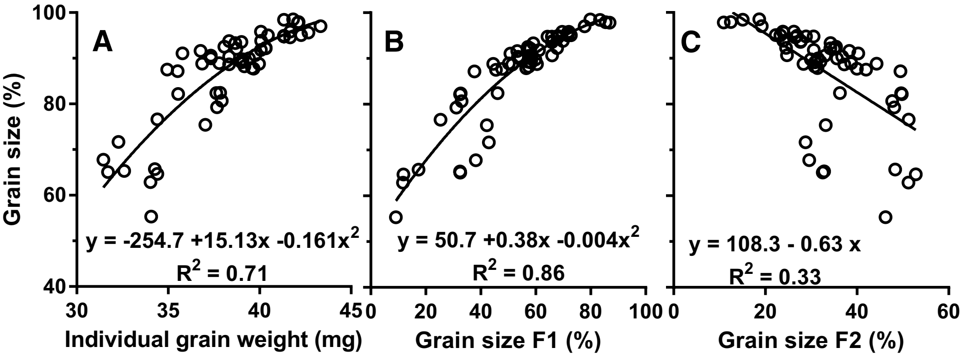


Fig. 1 Relationship between grain size (percentage > 2.5 mm) and **a** individual grain weight, **b** grain size fraction 1 (percentage > 2.8 mm) and **c** grain size fraction 2 (percentage between 2.5 and 2.8 mm). Each symbol represents a treatment mean at an experimental site. The line represents the fitted function. All fitted functions are significative ($p < 0.01$)

high (e.g., greater than 38 mg), variations of individual grain weight were associated with small variations of grain size, while when the individual grain weight was low, variations of individual grain weight were associated with higher variations of grain size. Variation in grain size was positively associated with variation in fraction 1 and negatively associated with fraction 2 (Fig. 1b, c). Consequently, when grain size fraction 1 increased, fraction 2 decreased (Eq. 3).

Grain size varied mostly due to differences between experimental sites and, to a lesser extent, due to treatments within each site. To analyze the variation in grain size due to treatments independently of the variation due to differences between environments, relative grain size was calculated by dividing the grain size of each treatment at a given site by the mean grain size of all treatments of that site. Similarly, relative fraction 1 and fraction 2 were calculated by dividing the value of each treatment at a given site by the mean value of that site. Relative grain size showed a close and positive association with relative fraction 1 and a weak and negative association with relative fraction 2 (Eqs. 4 and 5). These results suggest than grain size variation due to fertilizer treatments was determined by variation in grain size fraction 1.

$$\mathrm{F2} = -0.0064\,\mathrm{F1}^2 + 0.1585\,\mathrm{F1} + 45.460\left(\mathrm{R}^2 = 0.83;\ \mathrm{p} < 0.001;\ \mathrm{n} = 92\right) \tag{3}$$

$$\mathrm{GSrel} = 0.2823\,\mathrm{F1rel} - 0.7177\left(\mathrm{R}^2 = 0.78;\ \mathrm{p} < 0.001;\ \mathrm{n} = 92\right) \tag{4}$$

$$\mathrm{GSrel} = -0.0896\,\mathrm{F2rel} + 1.0896\left(\mathrm{R}^2 = 0.07;\ \mathrm{p} = 0.009;\ \mathrm{n} = 92\right) \tag{5}$$

Where F2 is grain size fraction 2 (% between 2.8–2.5 mm), F1 is grain size fraction 1 (% > 2.8 mm), GSrel is relative grain size, F1rel is relative grain size fraction 1, and F2rel is relative grain size fraction 2.

Grain Protein Concentration

In most experimental sites, grain protein concentration was low, with a minimum of 57 mg g^{-1} (Table 3). At only three sites, grain protein concentration in at least one treatment was higher than 125 mg g^{-1}, which is considered excessive by maltsters in Argentina. It should be stressed that genetic limitation for grain protein concentration was not very low as grain protein concentration above 154 mg g^{-1} was observed at one site. N fertilization significantly increased grain protein concentration in 18 of the 25 experiments. It is worth to note that grain protein concentration response to N fertilization was more frequent than yield response. The mean increase of grain protein concentration was 19 mg g^{-1} (calculated as the difference in grain protein concentration between N_0 and the highest value of N_1, N_2 or N_3). On average, for every kilogram of N applied grain protein concentration increased by 0.26 mg g^{-1} when N_1 and N_0 were compared and by 0.28 mg g^{-1} when N_1 and N_2 were compared.

Grain protein concentration in control treatments was not related to any environmental variable measured in this work. On the other hand, grain protein concentration response to N fertilization was negatively related to nitrate–N in the top 60 cm of the soil at sowing and positively related to N rate (Table 4). It is interesting that the absolute values of the parameters of both variables (N rate and nitrate–N) in the model were similar ($p = 0.97$). Grain protein concentration response was positively related to latitude: grain protein concentration increased by 0.22% for each degree of latitude. Latitude probably reflected another environmental variable directly related to grain protein concentration (like temperature or radiation), but we could not replace it by another variable in the model.

Grain protein concentration was negatively related to grain yield (Fig. 2). This relationship was not linear: when grain yield increased from 2 to 4 t ha^{-1} grain protein concentration decreased by 20 mg g^{-1}, while yield increases above 4 t ha^{-1} practically did not affect grain protein concentration. Grain protein concentration decrease as a consequence of grain yield increase was less than expected for a pure dilution effect. Grain protein concentration decreased along with the increase of the amount of N in grain per unit area (in Fig. 2, fitted function go through the lines of equal N per unit area).

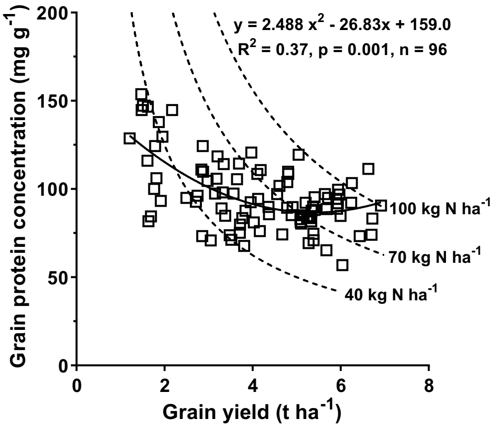


Fig. 2 Relationship between grain protein concentration and grain yield. Each symbol represents a treatment mean at an experimental site. The solid line represents the fitted function. Dotted lines connect points with equal grain nitrogen yield (grain nitrogen per unit area)

Grain protein concentration vs yield relationship, in our study, includes two sources of variations: (1) changes in N availability within each experimental site, and (2) differences in climatic, soil, biotic conditions, and crop management among experiments. Changes in grain protein concentration within each experiment were not associated with variations in grain yield (Fig. 3a). However, when variations among experimental sites were considered, changes in grain protein concentration were negatively associated with grain yield (Fig. 3b).

Within each experiment, grain protein concentration tended to increase with N fertilization, while when comparing among experiments, grain protein concentration tended to decrease with increased yield. These results suggest that grain protein concentration was a consequence of a balance between N availability and the demand of N for grain yield. To quantify this relationship, an index was calculated by dividing N availability by grain yield (Navail/GY). N availability was calculated as the sum of soil nitrate (0–60 cm) and fertilizer N. This ratio (Navail/GY) represented the kilograms of available N (soil + fertilizer) per tonne of grain yield, and the grain protein concentration was significantly associated with it (Fig. 4).

Discussion

Nitrogen Effects and Climatic Regulation of Yield, Grain Protein and Grain Size

Nitrogen fertilization effects on crop were consistent with that observed by other authors: increase in grain yield associated with an increase in grain number, increase protein concentration, decrease in grain size and, in a few

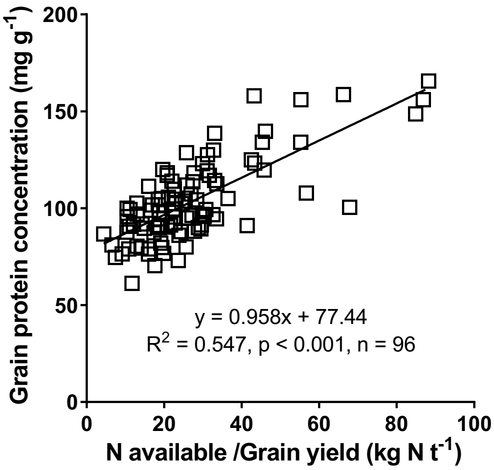
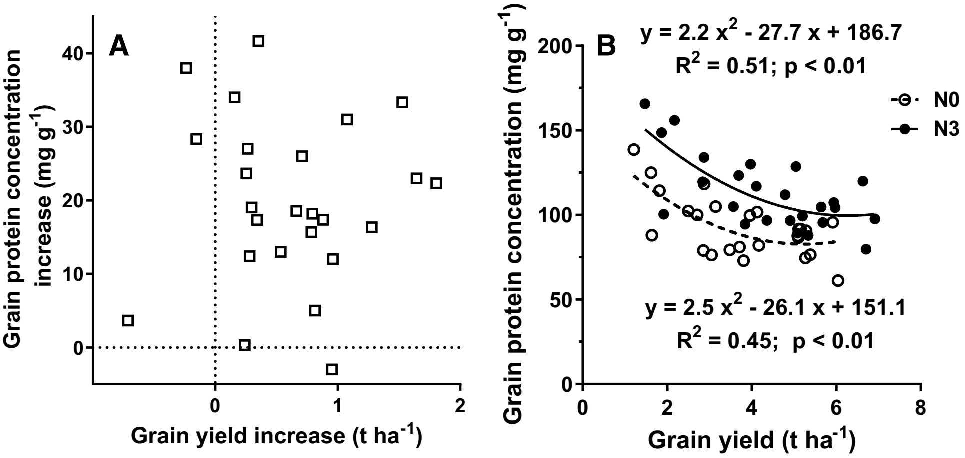


Fig. 4 Relationship between grain protein concentration and the ratio of N availability (the sum of soil N-nitrate up to 60 cm deep and N added as fertilizer) to grain yield. Each symbol represents a treatment mean in an experimental site. The line represents the fitted function

Fig. 3 **a** Relationship between grain protein concentration increase and grain yield increase (both calculated as the difference between the N_0 treatment and the N_{1-3} treatment with the highest yield) and **b** relationship between grain protein concentration and grain yield in treatments without (N_0) and with the highest N level (N_3). The lines represent the fitted function



cases, in individual grain weight (McKenzie et al. 2005; O'Donovan et al. 2011; Sainju et al. 2013).

Our results show that rainfall affects grain yield and grain protein concentration responses to N fertilization in a different way. In the Pampean region, as in other agricultural regions of the world, water availability is the most important factor affecting grain yield (Calviño and Sadras 2002; Verón et al. 2004). In our work, the effects of N fertilization on grain yield were conditioned by water availability: GYR model included the product of the rate of N fertilization and rainfall (full crop cycle). These results were consistent with the observations of Reussi Calvo et al. (2013) in wheat and of Abeledo et al. (2011) in barley. On the other hand, it is worth noting that the effects of N fertilization on grain protein concentration were not conditioned by water availability (rainfall was not included in the grain protein concentration response model). Then, drought tends to diminish N fertilization effects on yield but not N effects on grain protein concentration.

Grain yield and grain size in control plots were negatively associated with mean temperature during the critical period for grain number determination and with mean temperature during the grain filling period, respectively. These effects of temperature on grain yield and grain size could be interpreted as a consequence of the different period during the crop cycle when the two main yield components (number of grains and individual grain weight) are determined. While the critical period for grain number (and then for yield) is from 40 to 10 days before heading, the critical period for individual grain weight (and then for grain size) is during the grain filling period (Araus 2003; Arisnabarreta and Miralles 2008).

GYR was affected, also, by two soils characteristics: soil organic matter content and soil type. The negative association between GYR and organic matter it is likely to reflect N inputs from mineralization. On the other hand, the greater GYR in Argiudolls compared with other soil types could be a consequence of their high water holding capacity.

Grain Size and Individual Grain Weight

Grain size is one of the attributes of barley grain used to predict its quality for malting. It has long been known that malt extract (the main indicator of malt quality) is directly associated with individual grain weight and protein concentration of unmalted barley grains (Briggs 1998). Measuring individual grain weight is slow and tedious and, therefore, impractical for use during the marketing of barley (Briggs 1998). Moreover, grain size has shown a closer association with malt extract than individual grain weight (Mather et al. 1997; Bertholdsson 2004). These are the reasons why grain size has been widely adopted as a way to quickly assess the size distribution of grains.

In the scientific literature, the relationships between grain size and individual grain weight, and between grain size fractions were usually studied by comparing different cultivars. Our observations show that grain size and individual grain weight are positively associated when grains of the same barley cultivar but from different environments are compared. This association has been previously observed when different barley cultivars were compared (Mather et al. 1997; Passarella et al. 2003). In our study, when the individual grain weight was relatively high (e.g. greater than 38 mg), grain size was not very sensitive to changes in the individual grain weight.

The effects of treatments and environments on grain size were mostly explained by changes in the grain size fraction 1. Variations in fraction 1 was negatively associated with variations fraction 2, which is in agreement with the results reported by Fox et al. (2006) when comparing several malting barley cultivars.

Determination of Grain Protein Concentration

Grain yield seems to be a main factor in grain protein concentration determination: more the 50% of the grain protein concentration variance could be explained by the variation in yield. In cereals, the negative association between grain yield and protein concentration when different genotypes of the same species are compared is widely known (Slafer et al. 1990; Simmonds 1995; Barraclough et al. 2010). In his review, Simmonds (1995) noted that this relationship was weaker when comparing different environments than when comparing genotypes.

The negative association between grain yield and protein concentration, however, was not observed when grain yield varied due to changes in N availability within the same environment (e.g. when different levels of N were compared at a given experiment). Others authors have observed that when grain yield increased due to N fertilization, it may be accompanied by small decreases in grain protein concentration when N availability is very low, or by increases in grain protein concentration with medium to high N availability (Fischer et al. 1993; Lopez Bellido et al. 2004).

In our study, we could establish that grain protein concentration was determined by the ratio between N availability and grain yield, where N availability was assessed as the sum of N as nitrate in the soil at planting and N applied with fertilizers. The changes in grain protein concentrations within each experiment were explained mainly by variations in N availability, while changes in of grain protein concentration among experimental sites were mostly due to grain yield variations.

It is remarkable that, in this index, N availability includes three source of N with probable different efficiencies: N fertilization at sowing (north sites), N fertilization at tillering (south sites) and soil nitrate. In the model of grain protein concentration response to N, the absolute values of the parameters of N fertilization and soil N variables were similar (Table 4). This could indicate that N fertilizer average efficiencies are similar to soil N efficiency. Nevertheless, the good association between grain protein concentration and Navail/GY indicates that possible differences between N efficiencies were not extremely important.

Others authors have tried to explain the variations in grain protein concentration with indices reflecting the compromise between the accumulation of N and biomass in grains, though as far as we are aware, no one has used the one presented here. In a study of barley response to N fertilization conducted in Canada, changes in the grain protein concentration were related to the ratio of the N absorbed by the unfertilized crop plus applied N and the actual grain yield (McKenzie et al. 2004, 2005). Other authors have related grain protein concentration with the ratio between grain yield and absorbed N in wheat (Makowski et al. 1999; Barraclough et al. 2010) and between other crops (Sadras 2006). All these models assessed the supply of N by measuring N absorbed by the crop, information not available at the begging of the crop cycle. In our study we have used two components of the N supply from the soil (N as nitrate at planting and N applied as fertilizer).

The index proposed here has no predictive value as, when fertilizer is applied, the farmer does not know what would be the actual grain yield. However, this index has a practical value because it allows the farmer to analyze what are the possible combinations of grain yield and protein concentration that could be obtained with a given level of N availability. Moreover, using a realistic yield goal, this index could be used as a guide for making decisions on N fertilizer rates in order to obtain a desired grain protein concentration.

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