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Phosphorus, nitrogen, sulfur, boron and zinc fertilization effects on soybean yield and quality

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ABSTRACT

Seed protein concentration in argentine soybean has been steadily decreasing in the last decade and is causing severe losses to the soy milling industry. Thirty-one field trials were conducted in the Pampean region of Argentina during four seasons to evaluate the effect of different fertilization strategies- 1: Control without fertilization, 2: P (22 kg ha⁻¹); 3: P+S (22 kg ha⁻¹ P and 13 kg ha⁻¹ S); 4: PP+SS (40 kg ha⁻¹ P and 24 kg ha⁻¹ S); 5: P+S + Zn (T₃ + 1,25 kg ha⁻¹ Zn); 6: P+S + foliar B (T₃ + 0,1 kg ha⁻¹ B) and 7: P+S + foliar N (T₃ + 10 kg ha⁻¹ N) on soybean yield, seed protein concentration (PC), seed oil concentration (OC) and profat index (PF). In most site-years (58,1%), positive yield and protein responses were observed with PP+SS vs Control treatment, resulting in the largest response magnitudes compared to the rest of the treatments. Treatments with foliar N, preplant broadcast Zn and foliar B applications over T₃ had no consistent effects on the studied variables. This study shows that proper fertilization strategies can increase soybean yield and seed quality.

Introduction

Soybean industrial quality is mainly determined by its protein (PC) and oil concentrations (OC); PC is the protein content relative to other seed components such as oil and carbohydrates. The soybean milling industry competitiveness depends on soybean meal PC which must meet international standards to qualify as high protein and avoid price discounts and even rejections by customers. Argentine soybean PC has been steadily decreasing during the last decade causing severe losses to the milling industry (Cuniberti and Herrero, 2018).

Oil and protein synthesis and build-up processes in

soybean depend mainly on the genotype, the radiative and thermal environment during the grain filling stage, nutrient availability and crop yield (Thibodeau et al., 1975; Dardanelli et al. 2006; Rotundo and Westgate, 2009; Kane et al. 1997; Martinez and Cordone, 2015). Grain OC and PC are usually inversely related (Martínez and Cordone, 2015; Haq and Mallarino, 2005).

Soybean seed PC usually ranges between 33 and 42%, and its meal is one of the feeds with the highest protein value. A substantial portion of nitrogen (N) requirements in soybeans are met through soil absorption and nitrogen biological fixation (BFN) (Ronis et al., 1985).

In most cases, legume plants that obtain N through BFN have higher phosphorus (P), potassium (K) and sulphur (S) requirements than those plants that only depend on soil N (Israel, 1987; Sulieman et al., 2013).

Legumes are particularly sensitive to P, K and S deficiency. Divito and Sadras (2014) explain that these nutrients have a direct effect over BFN because they regulate multiple biological processes such as rhizobia growth and nodule formation and functionality. Pampean soils rarely lack K (Correndo et al., 2012; Sillanpaa, 1982) therefore, historically, this nutrient has not been included in field trials as a treatment. In fact, Prystupaet al. (2004) observed no maize yield response to K fertilization.

Numerous studies have shown soybean yield responses to P application when available P soil contents were low (Gutierrez Boem et al., 2006; Díaz-Zorita, 1999; Fontanetto et al., 2009; Ferraris et al., 2015; García et al., 2009; Aulakh et al., 1990). Sucunza et al. (2018) suggested a yield response threshold of 14 mg kg⁻¹ available soil P (Bray I). Regarding S, Carciochiet al. (2015) proposed a decision tree that suggests crop response to S fertilization with a threshold of 40 kg ha⁻¹ (approximately 5 mg kg⁻¹) of S-SO₄ in a 0-0,60 m depth, in soils with coarse texture, long agricultural history, low organic matter content, no-till sowing, no fallow and a water table without sulphates. Martinez and Cordone (2015) and Aulakh et al. (1990) reported positive effects of P and S fertilization on soybean grain PC. In contrast, Haq and Mallarino (2005) observed that P and K fertilization increased soybean yield but had little, infrequent and inconsistent effects on PC and OC. As Aulakh et al. (1990) explain, P is involved in the synthesis of phospholipids while S is required for oil storage organs which are proteinous in nature.

Under high yielding environments, BFN and N provided by soil organic matter could be insufficient to meet grain N demand during the filling stage. Foliar fertilization during late reproductive stages, when the number of pods has already been fixed, could theoretically increase N absorption, yield and PC. Moreira et al. (2017) observed 6.1% yield response when applying 10 kg

N ha⁻¹ at soybean growth stages R3 and R4. On the other hand, Salvagiotti et al. (2008) observed that foliar N fertilization increased yields from 130 to 620 kg ha⁻¹ under high yielding environments (over 4,5 Mg ha⁻¹). Barker and Sawyer (2005) found no response in soybean grain PC with soil N fertilization and foliar N fertilization in early reproductive crop stages. Moreira et al. (2017) obtained similar results as they did not find any effects of foliar N fertilization on PC and OC.

Micronutrients play an essential role in the plant metabolism (1995). Many studies observed that soybean yields and grain PC increased when B, Zn and Mg micronutrients were applied (Aytac et al., 2007; Bellaloui et al., 2010; Ghasemian et al., 2010; Panse, 2010).

The aim of this study was to evaluate the effect P, N, S, B and Zn fertilization on soybean yield and grain composition in order to assess a fertilization strategy that could increase PC and yield simultaneously.

Materials and methods

Experimental design

The experimental design was a randomized complete block with four repetitions, replicated in 31 site-years in the Pampean region of Argentina. Soybean varieties with maturity groups III to V were planted at a density of 300.000 seeds ha⁻¹ on 3 x 5 m experimental units (Table 1). Seeds were treated with fungicides and inoculated with *Bradyrhizobium japonicum* (except at one site-year where they were not inoculated). Weeds, pests and diseases were monitored and controlled when necessary. Soil samples were taken before planting at 0-0,20 m and 0,20-0,40 m depths to determine: pH (Peech, 1965), moisture, electric conductivity (Allison et al., 1977), organic matter (Nelson and Sommers, 1982), exchangeable cations (Richter et al., 1982), extractable P (Bray and Kurtz, 1945) and colorimetric quantification (Murphy and Riley, 1962), extractable Zn (Lindsay and Norvell, 1978), cations exchange capacity, nitrates (Marbán, 2005) and sulphates -ammonium acetate extraction at pH 5, soil:solution ratio 1:5 with one-hour agitation and turbidimetric quantification (Lisle et al., 1994) (Table 1).

Table 1. Soybean varieties, soil type, texture, pH, organic matter percentage (MO%), P-Bray and other soils variables in 31 site-years in the Pampean region in Argentina during years 13/14, 14/15, 15/16 and 16/17.

Site-Year	Soybean variety	Classification	Texture	pH	MO	NO ₃	N-NO ₃	P Bray	S-sulfates	S-sulfates	Zn	B	rainfall	rainfall	Sowing date	Clay/lime
					%	0-20cm	20-40cm		0-20cm	20-40cm			O+N+D	J+F+M		
2013																
1	DM 3810	Typic Argiudoll	ZS	6	5.9	31.2	16.5	5	4.9	4.1	1.8	N/A	162	414	1-nov	ND
2	NA 5009	Acuic Hapludoll	ZC	6.6	2.5	10.3	4.6	8.7	2.2	2.1	2.1	N/A	228	278	5-nov	ND
3	NA 5009	VerticArgiudoll	ZSL	5.7	3.1	16.9	8.6	7.4	3.8	2.4	0.5	N/A	281	633	16-nov	ND
4	DM 4800	Entic Hapludoll	ZS	6.4	1.9	3.7	1.5	11.6	2	2	0.9	N/A	133	246	6-nov	ND
5	DM 4210	Typic Argiudoll	ZS	6.3	5.2	17.6	9.7	5	3.3	2.6	2	N/A	194	328	23-nov	ND
6	DM 3810	Typic Argiudoll	Z	6	3.7	28.6	17.6	14.2	3.5	2	2.2	N/A	173	273	13-nov	ND
7	DM 3810	Typic Hapludoll	Z	6.4	1.9	13	2.4	11.6	2	3.7	0.9	N/A	205	370	2-nov	ND
8	DM 3810	Typic Hapludoll	ZS	6.3	2.1	5.9	2.2	10.9	5.6	2	0.7	N/A	417	375	30-oct	ND
2014																
9	HiPro 3010	Typic Hapludoll	Z	6	2.3	18	9	20.6	3.3	4.6	1	N/A	151	123	5-nov	19/33
10	DM 4670	Entic Hapludoll	ZS	6.4	1.6	9.2	7	13.3	4.5	1.5	0.7	N/A	253	182	5-nov	11.5/18
11	DM 4612	Typic Argiudoll	ZL	6	3.8	15.6	12.1	6.5	5.4	2.8	1.6	N/A	363	279	20-oct	24/49.5
12	FN 4,35	Typic Argiudoll	ZL	6.1	3.5	12.1	5.3	4.8	4.6	4.8	1.5	N/A	516	275	14-nov	11.5/55
13	ACA 4550	Entic Hapludoll	ZS	6.4	2.6	24	8.8	8.3	3.7	7.6	1	N/A	128	114	7-nov	16.5/28.5
14	SPS 3900 Plenus	Entic Hapludoll	ZSL	7.2	3.2	6.2	7	16.4	4.9	10	0.9	N/A	365	454	15-nov	21.5/41
15	SPS 3900	Entic Hapludoll	ZSL	6.6	4.8	3.5	2	20.5	11.1	2	1.5	N/A	365	270	10-oct	31.5/49.5
16	NA 5009	Typic Argiudoll	ZSL	6.1	3	8.1	4.2	5.9	2.6	2.2	0.7	N/A	363	361	8-nov	31.5/56.5
17	DM 4712	Typic Hapludoll	Z	6.6	2.5	14.7	11.9	11.4	2.1	N/A	2.2	N/A	228	174	27-oct	20/30
2015																
18	SPS RR 3x7	Thaptonatric Hapludoll	Z	6.3	1.9	7.0	4.0	9.1	2.5	2.5	N/A	0.5	322	242	4-nov	N/A
19	DM 4612	Typic Argiudoll	Z	6.1	3.1	21.6	9.5	5.8	3.1	2.8	N/A	1.2	154	260	16-nov	15/40
20	DM 4612	Typic Argiudoll	Z	6.2	3.3	7.5	5.3	20.5	4.4	4.5	N/A	0.7	413	460	23-oct	26.5/43.5
21	DM 3810	Typic Hapludoll	ZS	6.1	2.0	13.9	4.0	9.5	2.0	2.0	N/A	0.4	368	229	24-oct	12.5/18.5
22	DM 3810	Abruptic Argiudoll	Z	6.5	4.1	11.9	11	10.4	3.5	4.8	N/A	0.8	257	306	22-oct	27.5/59.5
23	DM 4212	Entic Hapludoll	Z	6.0	4.0	26.6	21.1	7.4	4.5	3.9	N/A	0.6	330	319	3-nov	15/40
24	DM 4915	VerticArgiudoll	ZSL	5.6	2.9	9.9	6.5	6.5	6.1	5.7	N/A	N/A	313	505	16-nov	N/A
25	DM 3810	Typic Hapludoll	ZS	6.1	1.9	11.4	6.8	10.4	2.7	1.5	N/A	0.6	301	461	21-oct	N/A
2016																
26	DM 4612	Typic Hapludoll	Z	6.5	1.6	21.1	7.5	4.1	3.4	N/A	0.3	0.4	428	426	4-nov	26.5/37
27	NA 5009	Typic Argiudoll	ZL	6	2.5	12.5	7.5	4.7	2.6	2	0.7	0.5	367	240	25-nov	24/63.5
28	ACA 4990	Abruptic Argiudoll	ZSL	6.1	2.7	14.7	12.1	3.4	2.7	3.8	0.6	0.5	432	409	10-nov	27.5/61.5
29	SPS 3x7	Typic Hapludoll	ZS	6.3	2.1	13.4	10.6	10.9	2.5	9.8	3.7	0.6	536	343	16-nov	17.5/22
30	DM 4670	Entic Hapludoll	ZS	6.6	2.1	9.5	3.5	6.9	2	2	0.9	0.5	470	195	11-nov	14/19.5
31	DM 4210	Typic Hapludoll	Z	6.1	3	21.1	14.3	8.6	4.6	5.4	1.8	0.5	394	310	6-nov	20/33.5

Z: silt; S: sand; C: clay; L: loam

The following treatments were repeated for four years: 1. Control without fertilization (T), 2. a replacement P rate of 22 kg ha⁻¹ -restores the P exported with the soybean seed for an estimated 4 Mg ha⁻¹ grain yield-, 3. treatment 2 + 13 kg ha⁻¹ of S at a replacement rate, and a double rate of 40 kg P ha⁻¹ + 24 kg S ha⁻¹ (PP+SS; Table 2). During the first year (2013) and one month before planting, monoammonium phosphate (Grade: 11: 22:0) was

broadcast as a source of P and Microessentials® was broadcast as a source of P and S (Grade: 12: 18: 0:10S). In this case, a base of 8 kg N ha⁻¹ was broadcast as urea in the control treatment to balance the N rates of the rest of the treatments. In the following years, calcium triple superphosphate (Grade: 0:20:0) was broadcast as the source of P, and simple superphosphate (Grade: 0:9:0:14S) was broadcast as the source of P and S.

Table 2. Nutrient rates applied to soybean crops for each treatment in 31 field trials in the Pampean region in Argentina.

Treatments	N	P	S	Zn	B	Trial Year
		----- kg ha ⁻¹ -----				
Control (T)		0	0			2013, 2014, 2015, 2016
P		22	0			2013, 2014, 2015, 2016
P+S		22	13			2013, 2014, 2015, 2016
PP+SS		40	24			2013, 2014, 2015, 2016
Foliar N	10					2013, 2014, 2015
Foliar B	0.3				0.1	2013, 2014, 2015
Zn		22	13	1.25		2013, 2014

Besides the aforementioned treatments, foliar N (Foliarsol U®), foliar B (Foliarsol B®) and Zn were applied at the typical rates used in the literature. Regarding foliar N, during the first year, 25 kg N ha⁻¹ were sprayed at R5[40], causing leaf burning in most site-years, so in the following years, a rate of 10 kg N ha⁻¹ was sprayed. Foliar B was sprayed at R2 at a rate of 100 g B ha⁻¹ (Fontanetto et al., 2009) during the last three years with a base fertilization of P and S (same as the rest of treatments), simulating a producer who adds technologies.

As for Zn fertilization, during two cropping years, 1.25 kg Zn ha⁻¹ were broadcast in a blend with P and S (*Microessentials Zn*® 12: 17: 0: 10S: 1Zn) before planting.

At the crop commercial maturity stage, 2m² of each plot were harvested by cutting the plants with garden scissors at ground level. Plants were sun-dried and threshed with a stationary thresher. Grain moisture was measured with a hygrometer and samples were weighed to calculate yield at a moisture of 13%. Then three groups of 200 seeds were counted and weighed

separately to calculate seed weight which was also expressed with 13% moisture.

Analytical methods

Five-hundred-gram seed samples from each plot were analysed with infrared spectrophotometry for PC and OC; results were expressed on a dry matter basis. The profat index (PF) indicates the industrial yield in the soybean crushing process and results from adding protein and oil percentages.

Statistical analysis

A mixed model which considered heterogeneous variances among site-years was used for the statistical analyses to find differences among treatments for yield, PC, OC and PF (SAS/STAT, 1992). Site-years were considered random factors while fertilization treatments were considered fixed factors. Pre-planned contrasts were performed to calculate the differences between treatments for each variable and their statistical significance, and to determine the standard errors for average values which were included in the figures. An alpha value of 0.1 was used to detect significant differences.

Results

P, P+S simple and double rates

Phosphorus and P+S treatments were repeated for four years; therefore, a four-year variance analysis was conducted. Each experiment was numbered by its year and site-year (alphabetically, data not shown) because not every site-year was repeated in all four years (Table 1).

Yield and yield components

The main effect of the broadcast pre-plant fertilization treatments was significant for yield

and its components ($P < 0,1$) while site-year x treatment interaction was not ($P = 0,343$); therefore, treatment values were averaged over all site-years (Table 3). The control treatment yields averaged 4 Mg ha^{-1} across years and the yield response to 22 kg P ha^{-1} averaged 261 kg ha^{-1} ($P < 0,001$). Adding 13 kg S ha^{-1} to the same P base increased yields another 163 kg ha^{-1} ($P = 0,034$), and a double rate of P+S increased yields 126 kg ha^{-1} over the simple P+S rate ($P = 0,086$). The yield responses were partly explained by a 5.3% increase in grain number caused by P application, and to a lesser extent to a 1.7% increase in seed weight caused by the combined P+S application (Table 3).

Table 3. Yield, seed weight, grain number and profat index of soybean seed for four fertilization treatments: T: control without fertilization; P: 22 kg P ha^{-1} ; P+S: 22 kg P ha^{-1} and 13 kg S ha^{-1} ; PP+SS: 40 kg P ha^{-1} and 24 kg S ha^{-1} in the Pampean region in Argentina during years 13/14, 14/15, 15/16 y 16/17. Different letters show significant differences for p-value < 0.1 within each variable.

Parameters	T	P	P+S	PP+SS	SE				
Yield (Mg ha^{-1})	4	d	4.3	c	4.5	b	4.6	a	0.06
Seed weight (g) $\times 1000$	169	c	170	bc	172	ab	173	a	0.88
Number of grains m^{-2}	2427	c	2556	b	2628	ab	2692	a	54
Profat index (%)	60.28	c	60.41	b	60.55	a	60.56	a	0.065
SE=standard error									

Protein concentration

Site-year x treatment interaction was significant for PC; therefore, results are shown for each site-year (Table 4). Protein concentration averaged 36.7% among control treatments, while it averaged 36.9% for P, 37.0% for P+S and 37.1% for PP+SS treatments. In four site-years, P application increased PC significantly by 1.6 percentage points (p.p.); P+S application increased PC at four site-years by 0.9 p.p. while the double rate application (PP+SS) increased PC at ten site-years by 1,5 p.p. over T. At one site-year, the PC response was negative for P and P+S applications (Table 4).

Yield and PC responses were plotted on a graph. Yield response was plotted on the X-axis and the PC response was plotted on the Y-axis (Fig. 1). At 41.9% of the site-years, positive yield and protein responses to P application were observed, averaging 217 kg ha^{-1} ($\text{SE} = 263$) and 0,22 p.p.

($\text{SE} = 0.36$, Fig. 1A), respectively. In addition, at 41.9% of the site-years, positive yield and protein responses to S application (with a P base) were observed, averaging 127 kg ha^{-1} ($\text{SE} = 400$) and 0.17 p.p. ($\text{SE} = 0.36$; Fig. 1D). Application of P+S and PP+SS compared to T determined that 51,6% and 58.1% of the site-years, respectively, had positive yield and PC responses (quadrant +,+; Fig. 1B and C). Yield average response on the positive quadrant was 314 kg ha^{-1} ($\text{SE} = 332$) for P+S (Fig. 1B) and 431 kg ha^{-1} ($\text{SE} = 395$) for PP+SS compared to T (Fig. 1C) while PC average response for the same quadrant was 0,31 p.p. ($\text{SE} = 0.4$) for P+S (Fig. 1B) and 0.48 p.p. ($\text{SE} = 0.5$) for PP+SS (Fig. 1C).

Oil concentration

Site-year x treatment interaction was significant for OC ($P = 0.072$). Oil concentration increased with the simple and double P+S rates in 16% of the site-years, but in 80% of the site-years, the response

was negative. In one site-year only, there was a positive response to P application (Table 5). Negative responses averaged -0.8, -0.5 y -1.4 p.p. with P, P+S and PP+SS treatments, respectively.

The OC decrease was associated with a significant PC increase (Pearson Correlation Coefficient $r = -0.80$; $P < 0.0001$). In five site-years, PC increased without an OC decrease.

Table 4. Protein concentration in soybean seed for four fertilization treatments T: control without fertilization; P: 22 kg P ha⁻¹; P+S: 22 kg P ha⁻¹ and 13 kg S ha⁻¹; PP+SS: 40 kg P a⁻¹ and 24 kg S ha⁻¹ in the Pampean region during years 13/14, 14/15, 15/16 and 16/17. Different letters show significant differences for p-value < 0.1 within each variable. SE= standard error.

Site	Control		P		P+S		PP+SS		SE
1	36.4	a	35.4	bc	35.1	bc	35.8	abc	0.2717
2	39.1		38.8		38.8		39.5		0.2717
3	39.3		39.4		39.7		39.1		0.2717
4	38.1		37.5		38.0		37.9		0.2717
5	34.5	b	34.7	b	34.1	b	35.9	a	0.2717
6	37.1		37.7		37.7		38.3		0.2717
7	35.8		35.0		35.6		35.1		0.2717
8	35.5		35.9		36.0		35.7		0.2717
9	43.4	c	46.3	a	43.5	c	45.3	b	0.2353
10	37.3	b	38.6	a	38.1	ab	38.7	a	0.2717
11	34.9		35.0		34.7		34.7		0.2717
12	38		37		38		38		0.2688
13	35.7	b	36.5	ab	36.3	ab	37.4	a	0.2717
14	36.9		36.4		36.6		36.9		0.2717
15	37		37		37		37		0.2688
16	36.8		36.7		37.5		37.5		0.2717
17	36.2		35.3		35.9		35.7		0.2717
18	34.7	b	36.3	a	36.3	a	35.8	a	0.2493
19	34		35		35		35		0.2353
20	36		36		36		36		0.2353
21	39.6		40.4		40.4		39.8		0.2353
22	38.6		38.2		37.7		38.0		0.2717
23	37.5	b	38.0	ab	38.2	ab	38.7	a	0.2353
24	34.4		34.4		35.5		35.0		0.2353
25	34.7		34.5		34.7		34.6		0.2353
26	33.2	b	34.0	b	35.2	a	35.5	a	0.2353
27	37.0	b	37.2	b	38.0	a	37.4	a	0.2353
28	34.9	c	36.0	b	36.4	bc	37.0	c	0.2353
29	36.1	b	35.7	b	36.6	b	37.1	a	0.2493
30	36.4		36.5		36.7		36.0		0.2353
31	38.2		38.1		38.0		37.7		0.2353

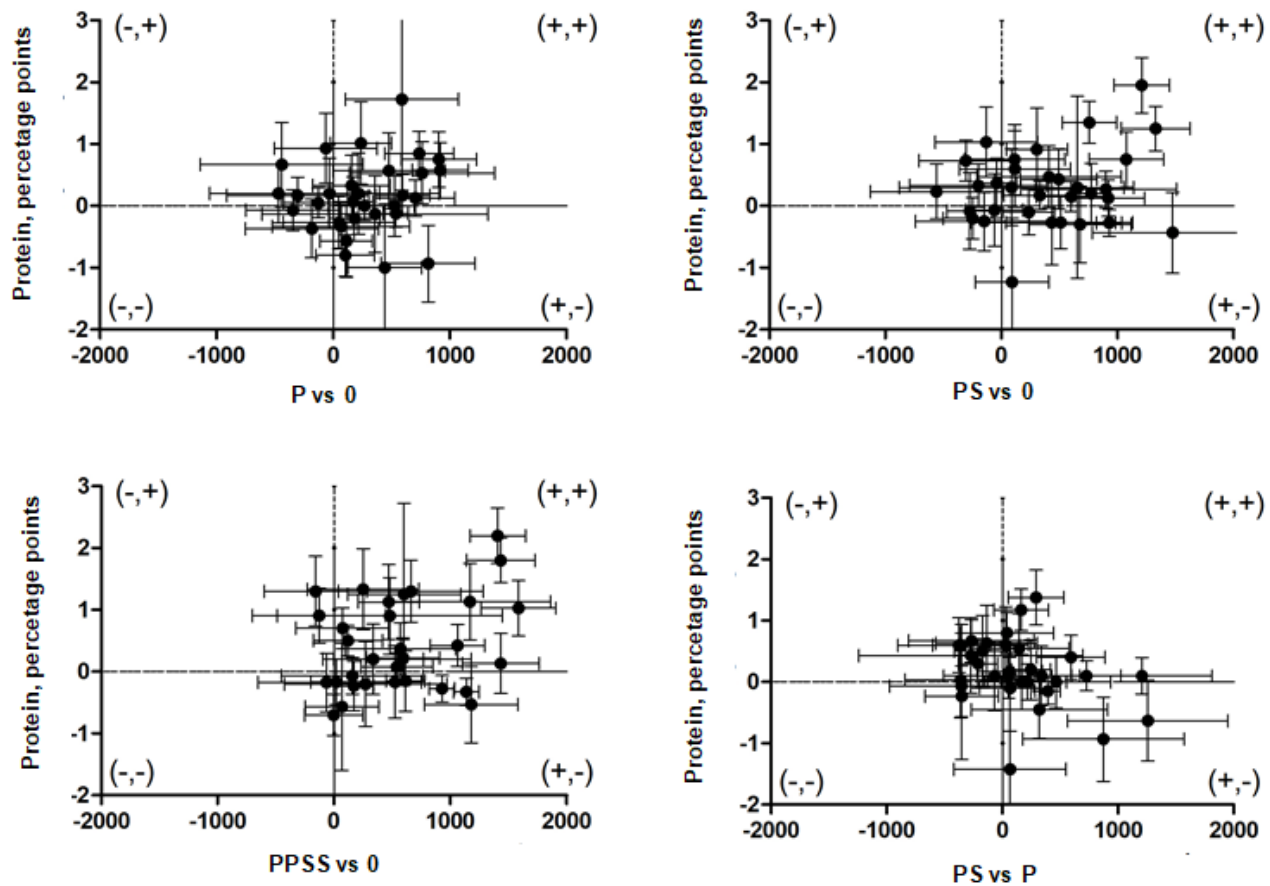


Fig. 1: Comparison of the relationship between yield (kg ha^{-1}) and protein (percentage points) response for different treatments: P vs 0 (22 kg P vs Control), P+S vs 0 (22 kg P and 13 kg S vs Control), PP+SS vs 0 (40 kg P and 24 kg S vs Control) and P+S vs P (22 kg P and 13 kg S vs 22 kg P) in the Pampean region in Argentina during years 13/14, 14/15, 15/16 and 16/17. Each point represents a different location with its standard error.

Profat

The fertilizer treatment main effect was significant for PF ($P < 0.1$). There was no treatment \times site-year interaction for PF; therefore, only main effects are shown (Table 3). Phosphorus, P+S and PP+SS treatments increased PF by 0.16, 0.29 and 0.34 p.p., respectively, compared to the control treatment, without significant differences between the simple and double P and S rates.

Zn fertilization effects

Preplant broadcast Zn applications had no significant effects on the measured variables despite the wide range of soil-available Zn concentrations (0.3 to 3.7 mg kg^{-1}).

Foliar N application at R5

Site-year \times foliar N treatment interactions were significant for yield and protein. In some site-years, foliar N application increased yield and PC, but in others, it had no effect or decreased them (Fig. 2). There was a tendency of an average decrease of 2.25 g in seed weight ($P = 0.12$) with 10 $\text{kg foliar N ha}^{-1}$ applied at R5. Oil concentration and PF were not affected by foliar N application.

Foliar B application at R2

There was no interaction between foliar B treatment and site-year for yield and seed weight. Foliar B application tended to increase seed weight by 2.37 g ($P = 0.11$), but this slight increase was not enough to produce a significant yield increase.

Table 5. Oil concentration in soybean seed for four fertilization treatments T: control without fertilization; P: 22 kg P ha⁻¹; P+S: 22 kg P ha⁻¹ and 13 kg S ha⁻¹; PP+SS: 40 kg P ha⁻¹ and 24 kg S ha⁻¹ in the Pampean region in Argentina during years 13/14, 14/15, 15/16 and 16/17. Different letters show significant differences for a p-value < 0.1 within each variable. SE= standard error.

Site	Control	P	P+S	PP+SS	SE				
1	23.2	23.8	23.7	23.5	0.1322				
2	21.7	21.7	21.7	21.7	0.1322				
3	21.7	21.7	21.7	21.9	0.1322				
4	24.3	24.1	23.9	24.2	0.1322				
5	24.3	24.4	24.6	24.4	0.1322				
6	22.4	22.1	22.2	22.2	0.1322				
7	24.1	24.4	24.1	24.2	0.1322				
8	24.4	24.4	24.5	24.5	0.1322				
9	18.1	a	16.6	c	17.4	b	16.9	b	0.1145
10	24.5	a	23.5	b	24.3	a	23.0	b	0.1322
11	24.8		25.0		25.0		25.2		0.1322
12	23.3		23.1		22.9		23.0		0.1301
13	24.7	a	24.7	a	24.7	a	23.4	b	0.1322
14	23.2		23.9		23.9		23.8		0.1322
15	22.7		22.8		22.8		22.7		0.1301
16	23.8		23.6		23.3		23.5		0.1322
17	21.7	b	23.0	a	22.9	ac	22.8	a	0.1322
18	24.0		23.8		23.8		23.2		0.1232
19	23.9		23.7		23.6		23.6		0.1145
20	23.6		23.9		23.9		23.9		0.1145
21	22.2		21.8		21.9		22.2		0.1145
22	24.4		24.1		24.4		24.6		0.1397
23	22.7		22.5		22.7		22.4		0.1145
24	23.6		23.6		23.5		23.3		0.1145
25	23.8		23.9		23.7		23.8		0.1145
26	27.0	a	26.2	b	25.7	bc	25.5	c	0.1145
27	23.4		23.2		22.7		23.2		0.1145
28	25.7		25.3		25.3		25.0		0.1145
29	23.9		24.0		24.1		24.0		0.1232
30	24.5		24.2		24.4		24.3		0.1145
31	24.8		24.9		25.0		25.0		0.1145

Regarding seed number, results were inconsistent. There was a significant interaction between the effect of foliar B with the site-year. At one site-year,

seed number increased by 218 seeds m⁻² (P=0.1) while at a different site-year, it decreased by 400 seeds m⁻² (P=0.023). At the rest of the site-years,

seed numbers were not modified. No effects of foliar B applied at R2 were observed on PC and PF. There was a foliar B treatment x site-year interaction for OC. At one site-year, OC decreased 0.25 p.p, while at a different site-year it tended to increase by 0.275 p.p. ($P=0.115$). At the rest of the

site-years, no effect of foliar B was observed on OC. Relationship between soybean yield (kg ha^{-1}) and seed protein (percentage points) response to 1.25 kg ha^{-1} Zn soil fertilization treatment in the Pampean region in Argentina during years 13/14 and 14/15 is depicted in Fig. 3.

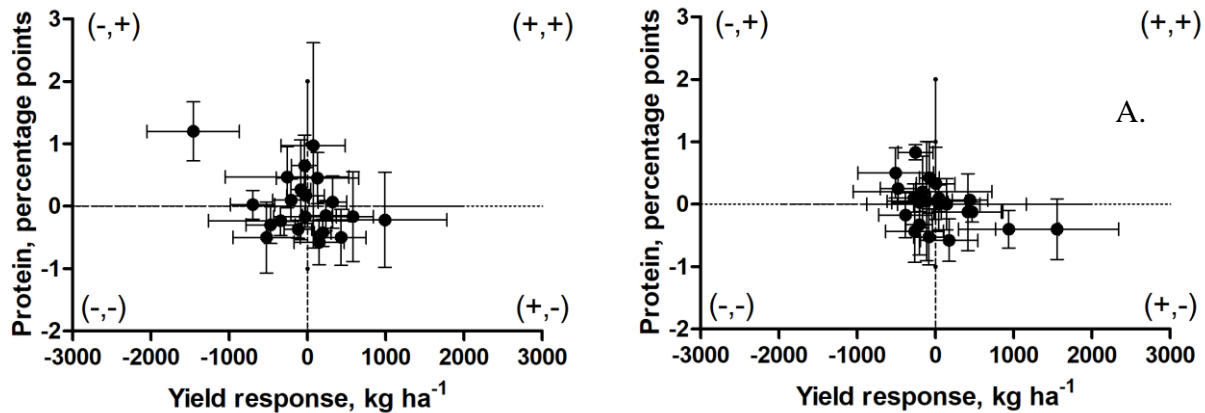
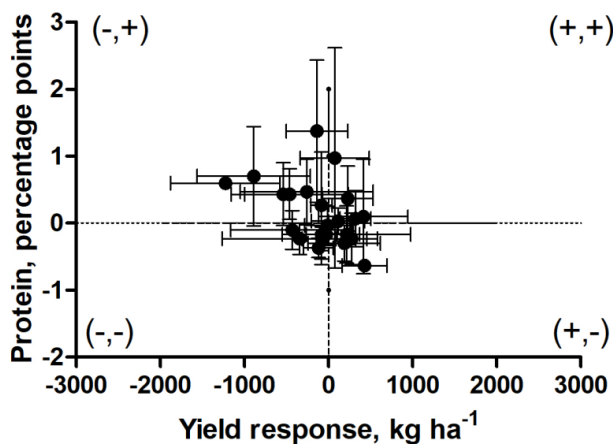


Fig. 2: Relationship between soybean yield (kg ha^{-1}) and seed protein (percentage points) response to A. 10 kg ha^{-1} N foliar treatment and B. 100 g ha^{-1} B foliar treatment in the Pampean region in Argentina during years 14/15, 15/16 and 16/17. Each point represents a location with its standard error.

Fig. 3: Relationship between soybean yield (kg ha^{-1})



and seed protein (percentage points) response to 1.25 kg ha^{-1} Zn soil fertilization treatment in the Pampean region in Argentina during years 13/14 and 14/15. Each point represents a location with its standard error.

Discussion

Yield response to P fertilization was consistent with soil P availability; in 83% of the site-years, soil analyses indicated P-Bray values under 14 mg

kg^{-1} , which is considered a response threshold by Sucunza et al. (2018). Yield response to S fertilization with a P base was not related to S-SO_4^{2-} soil levels or with soil organic matter content; this agrees with results observed by Salvagiotti et al. (2004) in experiments conducted on seven site-years in the northern Pampean region.

There was a clear trend towards increasing yield and PC with the application of P and S, especially when applied at a double rate (PP+SS). In effect, Aulakh et al. (1990), when evaluating soybean nutrition with P and S in a 3-year field experiment in India, found that a balanced P+S fertilization resulted in maximum seed yield and enhanced the process of protein and oil synthesis. In our experiments, P+S increased oil synthesis in kg ha^{-1} due to an increase in yield but had no effect OC.

Protein concentration and PF increases due to P+S application agree with Martínez and Cordone's (2015) observations in the Pampean region, as well as the positive response of OC at certain site-years and the negative response of OC at other site-years. In fact, Martínez and Cordone (2015) observed an even greater

number of site-years with positive PC and PF responses to P and S fertilization compared to our study. The negative response of OC is similar to the response reported by Haq and Mallarino (2005) in 112 field trials in USA.

The lack of response to Zn fertilization possibly resulted from an optimal root edaphic exploration and from a sufficient water availability that increases mobilization and transfer of nutrients from the soil to the crop (Rodriguez-Navarro et al., 2011). Besides, Sainz Rosas (2013) stated that greater Zn fertilization responses are expected when organic matter content is low and pH values are high. In our study, very few sites matched with this classification since organic matter ranged from 1.6 to 5.9 % and pH ranged from 5.6 to 7.2.

Foliar B application at R2 had no significant effect on yield, PC, OC or PF, possibly because, in most site-years, soil available B was close or above soybean sufficiency levels reported by several authors (Gupta et al., 1985; Datta et al., 1994; Cox and Kamprath, 1972). Only a slight increase in seed weight was found when foliar B was applied, which was also observed by Gambaudo et al. (2010) after applying foliar B in R3. In contrast to our study, Fontanetto et al. (2009) observed yield increases due to an enhanced pod set resulting from the application of 150 g B ha⁻¹ in R2-R3.

The lack of PC and OC response to foliar B was also observed by Fontanetto et al. (2009). However, in a glasshouse experiment, Bellaloui et al. (2019), observed an increase in nodule biomass, grain weight, yield and PC and a decrease in OC due to foliar B application in R2 working with potted Mollic Hapludalf soils brought from a field with an average 0.72 mg kg⁻¹ soil available B. No consistent effects were found on the studied variables with the foliar application of 10 kg ha⁻¹ N at R5; similar results were also observed by Moreira et al. (2017). On the contrary, Poole et al. (2010) applied foliar N solutions at R5-R7 and observed seed weight decreases, seed PC increases and OC decreases.

Conclusion

Phosphorus and P+S replacement rate fertilization produced a significant soybean yield increase, and a double P+S rate application produced an even

greater yield response. These treatments increased PC in many site-years while in most site-years where PC increased OC decreased. P and P+S simple and double rate treatments slightly increased PF. Pre-plant soil-applied Zn and foliar N and B applications had no effects on the studied variables. The foliar N treatment even caused phytotoxicity at some site-years, especially with rates above 10 kg N ha⁻¹.

This study shows that soybean yield and quality can be increased through agronomic practices such as fertilization with P and S, while the role of N and micronutrients over these factors remains unclear. A proper fertilization strategy could help increase farmers' incomes and milling industry competitiveness. Further research on protein building processes; particularly the effect of P and S nutrition on BNF and the contribution of BNF to seed protein concentration are needed to elucidate the macronutrient dynamics in the soybean plant metabolism.

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Conflict of interest statement

Authors declare that they have no conflict of interest.

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