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CIÊNCIAS AGRÁRIAS

Produção de sementes de soja de cultivares tolerante ou estável e cultivar suscetível ou responsiva à adubação fosfatada

Soybean seed production of tolerant or stable cultivars and susceptible or responsive cultivars to phosphorus fertilization

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RESUMO

O objetivo deste trabalho foi identificar os mecanismos responsáveis pelo desempenho superior de sementes de soja produzidas sob elevada disponibilidade de fósforo. Dessa forma, foi realizada a produção de sementes das cultivares DM 66i68 RSF IPRO (mais tolerante ou mais estável) e SYN 15630 IPRO (mais sensível ou responsiva) sob doses proporcionais a 0, 50, 100, 150 e 200% do recomendado de P para uma expectativa de rendimento de 5 t ha^{-1} de grãos. A adubação fosfatada promove maior crescimento de plântulas, melhoria de todos os componentes do rendimento, aumenta a proporção entre matéria seca total da parte aérea e a produção de grãos, aumenta a concentração dos minerais P, Ca, Mg, Fe e Zn. Sementes produzidas sob maior disponibilidade de fósforo promovem menor perda de solutos durante a embebição nos testes de condutividade elétrica e lixiviação de potássio e produzem plântulas com maior índice de velocidade de emergência, matéria seca de partes e área foliar.

Palavras-chave: Minerais; vigor de sementes; nutrição de plantas; crescimento de planta.

ABSTRACT

The objective of this study was to identify the mechanisms responsible for the superior performance of soybean seeds produced under high phosphorus availability. Thus, seeds of the DM 66i68 RSF IPRO (more tolerant or more stable) and SYN 15630 IPRO (more sensitive or responsive) cultivars were produced at proportional P rates of 0, 50, 100, 150, and 200% of the recommended yield for an expected grain yield of 5 t ha-1. Phosphate fertilization promotes greater seedling growth, improves all yield components, increases the ratio of total shoot dry matter to grain yield, and increases the concentration of P, Ca, Mg, Fe, and Zn. Seeds produced under higher phosphorus availability promote less solute loss during imbibition in electrical conductivity and potassium leaching tests and produce seedlings with higher emergence speed, shoot dry matter, and leaf area.

***Keywords**: Minerals; seed vigor; plant nutrition; plant growth.*

1. INTRODUCTION

The production of soybean seeds with doses higher than those used during grain production has already been carried out empirically by several seed producing companies in Brazil. However, despite the existence of many research data that have observed improvement in seed performance under greater availability of phosphorus, the results are variable among themselves. On the other hand, there are few results on the performance of cultivars in phosphorus-limiting situations. (Batistela Filho et al., 2023; Marin et al., 2020; Oliveira, 2020; Pazzin, 2021).

There are beneficial effects on viability and vigor by the tetrazolium test (Marin et al., 2020) and on seedling emergence (Pazzin, 2021) of soybean seeds produced under high levels of phosphorus fertilization. However, these tests are more general and do not identify the possible causes of higher seed vigor due to phosphorus. In addition, an effect on the physiological quality of seeds is often not identified when the evaluation is carried out by tests most frequently used, such as germination and field emergence tests (Marin et al., 2020; Batistella Filho et al., 2023).

Among plant responses to phosphorus limitation is the replacement of phospholipids in plant membranes by glycolipids and sulfolipids to save P and maintain membrane integrity (Plaxton & Tran, 2021). If this replacement process also occurs in soybean seeds, considering that glycolipids and sulfolipids may be less effective than phospholipids in maintaining the integrity of seed membranes,

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it may generate higher rates of solute leaching during seed soaking, reducing seed vigor. Electrical conductivity and potassium leaching tests, due to their relationship with membrane integrity, are potentially capable of detecting variations in this characteristic. Batistella Filho et al. (2023) did not observe the effect of phosphate fertilization on electrical conductivity and found an increase in potassium leaching under higher doses of P, however, the seeds used by these authors were produced in the field, where several factors can affect quality.

The objective was to evaluate the effect of phosphorus fertilization on the development of plants in the field and the effects on the physiological quality of cultivars with contrasting performance in terms of response to phosphorus fertilization.

2. DEVELOPMENT

The work was carried out in a greenhouse and at the Seed Analysis Laboratory of the Graduate Program in Seed Science and Technology, School Agronomy Eliseu Maciel, Federal University of Pelotas. The correction and fertilization of the soil was carried out considering the result of the chemical analysis, for an expected productivity of 5 tons ha-1, with the exception of the different doses of phosphorus, which were carried out according to each treatment, following the technical recommendations (CQFS, 2016).

Liming and fertilization with P and K were incorporated prior to sowing, using calcium oxide, ground triple superphosphate and ground potassium chloride, respectively, as sources.

Considering results obtained in preliminary evaluations and similarities regarding the maturation group (MG), the cultivars DM66i68 RSF IPRO (MG 6.6), identified as more tolerant or more stable, and SYN 15630 IPRO (MG 6.3) classified as more susceptible or responsive were chosen. Five doses of phosphorus added to the soil were used, corresponding to 0, 50, 100, 150 and 200% of the recommended dose of P. The soil presented P content classified as very low and clay class 4, with the supply of 100% correction and maintenance fertilization.

Sowing was carried out in polyethylene buckets, with a capacity of 20 liters, filled with 24 kg of dry and sieved soil from the A1 horizon of an Albaqualf Soil used as substrate. The seeds were previously treated with Standak® Top (fungicide and insecticide). Inoculation was performed together with sowing, using peat inoculant containing the species *Bradyrhizobium japonicum*, verifying the efficiency of nodulation 12 days after emergence.The following evaluations were carried out:

2.1 Morphological characteristics

Determined after 30 days, at regular intervals of 15 days. Plant height was determined using a graduated ruler, measuring the distance between ground level and the upper end of the stem. The stem diameter was determined with the aid of a digital caliper and expressed in mm and the number of nodes through direct counting, considering those in which the emission of completely expanded leaves occurred. At the end of cultivation, the plants of each repetition were cut close to the base of the soil and the dry matter of the main branch (DMMB), branches (DMB), pods (DMP) were determined. The shoot mass (DMSM) was calculated from the sum of DMMB, DMB, DMP and grain yield. Dry matter of leaves and roots was not determined due to their high degree of deterioration

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at harvest. Results for variables are presented in grams. Additionally, the percentage between grain yield and DMSM (GPA) was calculated.

2.2 Yield componentes and seed yield

The number of pods, seeds per pod and the mass of a thousand seeds were evaluated. Grain yield (g plant^{-1}) was determined by manually harvesting and threshing the pods of all plants of each treatment followed by moisture correction to 13%. From the fraction harvested in each experimental unit, a uniformity test was performed (retention on sieves), determining the predominant seed size in each treatment. Additionally, with the weighted average between the percentage of seeds retained in each sieve and the weight of each sieve diameter, the approximate value of the average seed size was obtained. Subsequently, non-standard seeds (seeds smaller than 5.0 mm) were discarded. The predominant seed size was used to obtain grain production generation, using seeds of the same size for all treatments.

2.3 Chemical composition of seeds

Determined by acid digestion of samples, followed by elemental determination by ICP-MS. The acid digestion was performed using approximately 0.1 g of the samples, which were weighed in 15 mL tubes (falcon type), adding 1.5 mL of concentrated HNO3. The samples were kept at rest for 24 hours for pre-digestion. They were then placed in a water bath, where they were left for 4 hours at a maximum temperature of 90 °C. Ultrapure water was used for measurement (15 mL) and the solutions were stored for later analysis by ICP-MS. Some reference materials were used in order to verify the accuracy and precision of the sample preparation method used.

2.4 The materials used were

NIST 1640a (natural water) and NIST 1568b (rice flour), both are certified reference materials from the National Institute of Standards and Technology (NIST, USA). In addition to these, two internal reference materials were also used to assess accuracy: one material made from soy flour and the other from rice flour. Duplicates of each reference material were subjected to the same procedure as the samples. After digestion, the samples were analyzed in a mass spectrometer (ICP-MS 7900, Agilent, Hachioji, Japan) equipped with a reaction cell to minimize spectral interference. Internal standard of Y (10 ppb) was used during the analysis to evaluate the equipment response. The limits of detection and quantification of the method were calculated according to the instructions described in the analytical validation protocols of Metrology, Quality and Technology National Institute (INMETRO/BRAZIL).

2.5 Germination test (G) and first germination count (FGC)

The 4 subsamples of 50 seeds per experimental unit were used, sown in Germitest® paper rolls moistened with an amount of water equivalent to 2.5 times the weight of dry paper, remaining in a germinator at 25°C. The evaluations were performed at five (FGC) and eight (G) days after sowing (Brasil, 2009), and the results expressed as a percentage of normal seedlings.

2.6 Electrical conductivity and potassium leaching

Used 4 subsamples of 25 seeds per experimental unit were used. The seeds were weighed and pre-soaked for 12 hours in a gerbox to perform the accelerated aging test. After this period, the seeds

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were weighed again and transferred to plastic cups with a capacity of 200 mL of total volume, where they received 75 mL of deionized water. The pre-soaking and soaking periods were carried out in a room at 20°C. Readings were taken after four, eight and 24 hours of imbibition, homogenizing the sample for reading. After this period, the electrical conductivity of the solution was determined using a Digimed conductivity meter (model MD-31). The results were expressed in μS cm^{-1} g^{-1} of seed. Simultaneously with the conductivity reading, 1 mL aliquots of each sample were collected for the potassium leaching test. The solute was diluted in 10 mL of deionized water and the potassium concentration was determined by reading it in a MICRONAL® flame photometer (Model: B462, São Paulo, SP, Brazil). The results were expressed in mg of potassium per g of seed.

2.7 Field emergence in the

Used 200 seeds were used, divided into four subsamples of 50 seeds, sown in Albaqualf Soil, at a depth of 3 cm, evaluated 15 days after sowing. The experiment was conducted in complete randomized blocks with 4 replications, in a factorial scheme, cultivars (tolerant and susceptible [2]) x phosphorus doses (0, 50, 100, 150 and 200% of the recommended dose [5]). Statistical analyzes were performed using R (R Core Team, 2020), through the RStudio interface (RStudio Team) and the ExpDes.pt statistical package (Ferreira et al., 2014). The data were subjected to analysis of variance and, when the calculated F was significant at a 5% probability level and the assumptions of the analysis of variance were met, the averages of the cultivars were compared using the t test, for the dose effect, the most representative polynomial through regression analysis.

3. RESULTS AND DISCUSSIONS

There was an effect of the factor cultivar and dose in all periods evaluated on the variables number of nodes and plant height, except for number of nodes at 60 days after sowing (DAS) and plant height at 75 DAS where there was only the simple effect of dose of phosphorus. For stem diameter there was interaction between the factors for all evaluated periods.

Cultivar SYN 15630 IPRO had the highest number of nodes while cultivar DM 66i68 RSF IPRO was superior for plant height (Table 1). Table 2 presents the results for stem diameter of each cultivar at each evaluated dose. Phosphate fertilization led to more pronounced responses for the SYN 15630 IPRO cultivar.

Table 1 - Morphological characteristics of soybean plants of stable (DM66i68 RSF IPRO) and responsive (SYN 15630 IPRO) cultivar to phosphorus fertilization.

Cultivar	N_30	N_45	N_75	A_30	A_45	A_60
DM 66i68 RSF IPRO	7.49 b	11.33 b	17.55 b	31.84 a	54.39 a	83.26 a
SYN 15630 IPRO	7.74 a	11.65 a	18.27 a	24.96 b	45.39 b	76.41 b
Average	7.62	11.49	17.91	28.40	49.89	83.57

* Means that do not share a letter are significantly different by t test. For the N and A symbols, present in the table above, the description of N means number of nodes while A means plant height.

Source: Gustavo Zimmer.

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Table 2 - Diameter of the stem of soybean plants of the (DM66i68 RSF IPRO) and responsive (SYN 15630 IPRO) cultivar to phosphorus fertilization.

Evaluation period (days)	Cultivar	Doses of Phosphorus (%)									
		0		50		100		150		200	
		Mm									
30	DM 66i68 RSF IPRO	4.32	ns	6.08	ns	6.30	b	7.13	ns	6.95	b
	SYN 15630 IPRO	4.12		6.01		6.86	a	7.00		7.23	a
45	DM 66i68 RSF IPRO	6.05	ns	8.44	ns	8.55	b	9.61	ns	9.10	b
	SYN 15630 IPRO	5.73		8.64		9.33	a	9.31		10.13	a
60	DM 66i68 RSF IPRO	6.67	ns	9.69	ns	10.17	ns	11.18	ns	10.49	b
	SYN 15630 IPRO	6.39		10.11		10.56		11.49		11.77	a

* Means that do not share a letter are significantly different by t test at 5% error probability.

Source: Gustavo Zimmer

There was interaction between the cultivar and phosphorus dose factors for all variables except GPA where there was a significant effect for the isolated main factors. The non-significant interaction for the GPA variable and the highest value observed for the variable in the SYN 15630 IPRO cultivar (Table 3) demonstrate that greater dry matter production during early development under P-limiting conditions does not necessarily lead to greater production of grain. The selection of cultivars with greater tolerance to phosphorus deficiency will possibly obtain better results if selection based on grain yield under limiting conditions is used as a strategy.

Table 3 - Percentage of grains in relation to the dry matter of the components of the area part (GPA).

Cultivar	GPA (%)
DM 66i68 RSF IPRO	43.77 b
SYN 15630 IPRO	48.60 a
Overall Average	46.19

* Means that do not share a letter are significantly different by t test at 5% error probability.

Source: Gustavo Zimmer

For yield components, it was observed that there was interaction between cultivar and dose factors for the variables number of pods, pods with 2 grains, pods with 3 grains, grains per plant and grain yield. As for the variables pods with 1 grain, grains per pod and weight of a thousand seeds (PMS), the simple effect of the factors was observed. Finally, for the empty pods' variable, there was no effect of any of the factors on the response variable.

The averages for the variables that showed a simple effect of cultivar and absence of interaction are presented in Table 4. The cultivar DM 66i68 RSF IPRO presented, on average, a higher percentage of pods with one grain and a higher weight of a thousand seeds, on the other hand, the cultivar SYN 15630 had the highest number of grains per pod. Cultivar SYN 15630 IPRO showed superior results to DM 66i68 RSF IPRO for all variables at the dose of 200% P, except for the percentage of pods with 2 grains, being superior in all doses for the variable pods with 3 grains (Table 5).

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Table 4 - General averages of cultivars for yield components pods with one grain, and grains per pod and weight of a thousand seeds (WTS).

Cultivar	Pods with one grain (%)	Grains per pod	WTS (g)
DM 66i68 RSF IPRO	25.42 a	1.74 b	155.62 a
SYN 15630 IPRO	14.02 b	1.99 a	148.36 b
Average	19.72	1.87	151.99

* Means that do not share a letter are significantly different by t test at 5% error probability.

Source: Gustavo Zimmer

Table 5 - Yield components in a stable and responsive cultivar to phosphorus fertilization.

Response variable	Cultivar	Doses of Phosphorus (%)									
		0		50		100		150		200	
		Mm									
Number of Pods	DM 66i68 RSF IPRO	11.29	a	53.08	a	63.33	a	81.83	a	76.79	b
	SYN 15630 IPRO	10.13	a	44.33	b	64.83	a	72.20	b	85.04	a
2 grain pods	DM 66i68 RSF IPRO	54.23	b	69.04	a	69.26	a	72.30	a	70.66	a
	SYN 15630 IPRO	71.89	a	68.24	a	65.52	a	70.80	a	70.44	a
3 grain pods	DM 66i68 RSF IPRO	0.81	b	4.46	b	5.60	b	6.54	b	6.63	b
	SYN 15630 IPRO	7.52	a	15.47	a	21.04	a	19.51	a	19.99	a
Grains per plant	DM 66i68 RSF IPRO	17.42	a	92.67	a	112.3	b	152.2	a	139.4	b
	SYN 15630 IPRO	18.58	a	86.96	a	131.8	a	151.5	a	177.5	a
Plant Production	DM 66i68 RSF IPRO	2.52	a	12.80	a	17.38	a	26.36	a	24.60	b
	SYN 15630 IPRO	2.40	a	12.43	a	21.75	a	25.69	a	30.23	a

* Means that do not share a letter, in a same variable, are significantly different by t test at 5% error probability.

Source: Gustavo Zimmer

The percentage of seeds on the 7 and 5 mm sieves showed interaction between the cultivar and dose factors, while the percentage of seeds retained on the 6.5, 6.0, 5.5 sieves and average seed size there was only the simple effect of the factors.

In Figure 1 it is possible to observe that the increase in phosphorus availability increased the percentage of seeds retained in sieves 7.0 (A) and 6.5 (B) and in the average size of the seeds, while for the seeds retained in sieves 6.0, 5.5 and 5.0 there was a reduction in the observed values because of greater availability of phosphorus. Seeds retained on sieves 5.0, 5.5 and 6.0 had higher percentages for the SYN 15630 IPRO cultivar (Tables 6 and 7). A greater increase in the percentage of seeds retained on the 7.0 mm sieve was observed in the cultivar DM 66i68 RSF IPRO (Table 7).

Table 6 - General averages of cultivars for the percentage of seeds retained in each sieve (S) and seed size.

Cultivar	S 5.5 mm (%)	S 6.0 mm (%)	S 6.5 mm (%)	General averages (mm)
DM 66i68 RSF IPRO	10.27 b	37.70 b	45.00 a	6.19 a
SYN 15630 IPRO	16.27 a	45.07 a	32.33 b	6.09 b
Average	13.27	41.38	38.66	6.14

* Means that do not share a letter are significantly different by t test at 5% error probability.

Source: Gustavo Zimmer

Table 7 - Percentage of seeds retained on 5 and 7 mm sieves (S) for seeds of a stable (DM66i68 RSF IPRO) and responsive (SYN 15630 IPRO) cultivar to phosphorus fertilization.

Response variable	Cultivar	Doses of Phosphorus (%)									
		0		50		100		150		200	
		mm									
S 5 mm	DM 66i68 RSF IPRO	2.91	b	4.04	a	0.48	a	0.39	a	0.10	a
	SYN 15630 IPRO	7.85	a	2.76	a	0.60	a	1.04	a	0.50	a
S 7 mm	DM 66i68 RSF IPRO	0.40	a	0.92	a	3.26	a	9.30		12.63	a
	SYN 15630 IPRO	0.81	a	0.13	a	3.69	a	7.25		5.91	b

* Means that do not share a letter, in a same variable, are significantly different by t test at 5% error probability.

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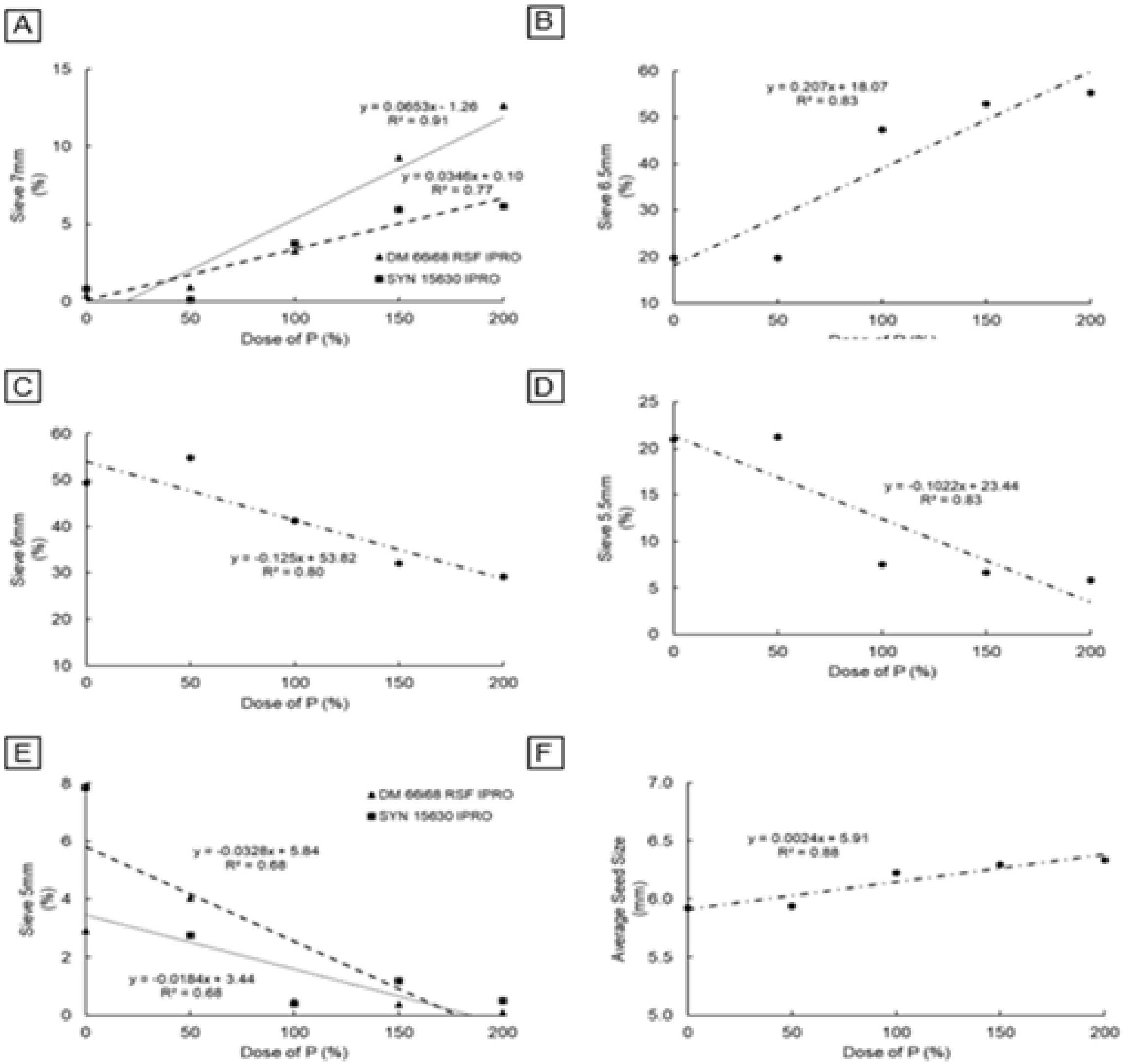
Figure 1 - Seed retention of cultivars stable and responsive to phosphorus availability under different doses of P.



Source: Gustavo Zimmer

Despite the interaction between cultivar and dose for the percentage of seeds retained on the 7.0- and 5.0-mm sieves, there was no interaction for the weighted average of seed size, which indicates that phosphorus availability leads to an increase in seed size at a similar intensity regardless of the grow crops.

Excessively large seeds are more susceptible to mechanical damage during harvesting and processing and require a greater weight of seeds per sowing area. In the case of selling seeds by number, larger seeds are less profitable for the seed producing company and in a situation of selling seeds by weight, the cost of sowing becomes higher for the rural producer. These considerations show that the commercialization of seeds produced under high doses of phosphorus needs to include value-added marketing in order to be commercially justified. This value-added marketing is most advantageous in cultivars that have naturally smaller seeds.

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As for the macronutrients (table 8), it is observed that in the absence of phosphorus fertilization (0%) the cultivar SYN 15630 showed a higher concentration of phosphorus in the seeds, while in the doses 150 and 200% the concentration of phosphorus was higher for the cultivar DM 66i68 RSF IPRO. These results are congruent with the results observed by Marin et al. (2020) who observed increases in P concentration in the seeds of the CD 242 RR cultivar that fit a quadratic model. Singh et al. (2019) also observed an increase in the concentration of P in soybean seeds produced under higher concentrations of P. The concentration of P in the seeds of the experiment ranged from 3.18 to 5.92 g of P kg of seed^{-1}, lower than the amounts observed by Marin et al. (2020), between 6 and 8 g of P Kg of seed$^{-1,}$ within the limits observed by Singh et al. (2019) which remained between 3 and 8 g of P kg of seed^{-1}.

Potassium concentrations (Table 8) fitted in an increasing linear model for the DM 66i68 RSF IPRO cultivar, and for the SYN 15630 IPRO cultivar the K concentrations fitted a decreasing model. The observed levels of the nutrient varied between 17.08 and 20.07 g of K kg of seed^{-1}, a level similar to that observed by Marin et al. (2020) and Singh et al. (2019). Although the levels are similar, the observed trends differ from the results obtained by Marin et al. (2020) who obtained a quadratic behavior for the nutrient, with an initial reduction in the K concentration for the doses of 50, 100 and 150% of P followed by a marked increase in the dose of 200%. In contrast, Singh et al. (2016) observed an increase in the concentration of K in seeds of the Spencer cultivar as a result of a greater supply of P.

Table 8 - Concentration of macro and micronutrients in soybean seeds in stable (DM66i68 RSF IPRO) and responsive (SYN 15630 IPRO) cultivar to phosphorus availability under different doses.

Nutrient	Cultivar	Doses of Phosphorus (%)					Averages
		0	50	100	150	200	
		g of nutrient per kg of seeds					
P	DM 66i68 RSF IPRO	3.18 b	3.43 a	3.74 a	4.53 a	5.92 a	4.16
	SYN 15630 IPRO	3.74 a	3.55 a	4.04 a	3.94 b	4.77 b	4.01
K	DM 66i68 RSF IPRO	17.08 b	18.24 a	18.60 a	18.82 a	19.87 a	18.52
	SYN 15630 IPRO	20.07 a	19.36 a	19.09 a	17.61 a	17.77 b	18.78
Ca	DM 66i68 RSF IPRO	2.56	2.83	2.80	2.73	2.91	2.77 b
	SYN 15630 IPRO	3.02	3.09	3.35	3.20	3.36	3.20 a
Mg	DM 66i68 RSF IPRO	1.95 a	2.10 a	2.14 a	2.27 a	2.63 a	2.22
	SYN 15630 IPRO	1.91 a	2.08 a	2.20 a	2.04 b	2.18 b	2.08
S	DM 66i68 RSF IPRO	3.44 b	3.38 a	3.43 a	3.38 a	3.59 a	3.44
	SYN 15630 IPRO	4.06 a	3.60 a	3.43 a	3.14 a	3.19 b	3.48
		mg of nutrient per kg of seeds					
B	DM 66i68 RSF IPRO	37.26	32.63	33.37	31.27	30.22	33.04 a
	SYN 15630 IPRO	35.31	30.80	29.62	26.39	27.02	29.83 b
Co	DM 66i68 RSF IPRO	0.39 a	0.54 a	0.19 a	0.17 a	0.22 a	0.30
	SYN 15630 IPRO	0.30 a	0.44 a	0.22 a	0.19 a	0.22 a	0.27
Cu	DM 66i68 RSF IPRO	5.49 a	4.96 a	4.53 a	3.48 a	3.58 a	4.41
	SYN 15630 IPRO	5.69 a	4.61 a	3.59 b	2.90 a	2.28 b	3.81
Fe	DM 66i68 RSF IPRO	50.21 b	60.16 a	57.53 b	58.47 b	62.51 b	57.78
	SYN 15630 IPRO	60.01 a	62.58 a	70.66 a	71.03 a	76.49 a	68.15
Mn	DM 66i68 RSF IPRO	126.14 b	103.47 a	94.93 b	85.27 a	102.97 a	102.55
	SYN 15630 IPRO	203.64 a	121.59 a	127.03 a	101.71 a	102.88 a	131.37
Mo	DM 66i68 RSF IPRO	2.14	0.55	0.15	0.06	0.05	0.61 b
	SYN 15630 IPRO	2.79	0.79	0.27	0.11	0.09	0.84 a
Ni	DM 66i68 RSF IPRO	2.42 a	3.01 a	2.76 a	2.26 a	3.25 a	2.74
	SYN 15630 IPRO	2.52 a	3.24 a	3.07 a	2.39 a	2.48 b	2.74
Zn	DM 66i68 RSF IPRO	28.77 b	30.36 a	31.63 a	32.43 a	33.91 a	31.42
	SYN 15630 IPRO	32.79 a	31.16 a	32.21 a	30.86 a	31.80 a	31.77

*Means that do not share a latter, in a same variable, are significantly different by t test at 5% error probability.

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Cultivar SYN 1530 IPRO differed from DM 66i68 RSF IPRO with higher calcium concentrations (Table 8) similarly to the data obtained by Singh et al. (2019), who observed an increase in Ca concentration at higher doses of P. On the other hand, they contrast with the result obtained by Marin et al. (2020) who observed a downward trend in the content of this nutrient.

Additionally, this research used a lower range of P_2O_5 doses and a soil with a higher initial phosphorus and clay content (0 – 108.68 kg ha^{-1}, 11.5 mg of P dm^{-3} and 30%, respectively) comparatively to this work (0 – 506 kg ha^{-1}; 3.7 mg of P dm^{-3} and 14% of clay). Soils with a higher clay content have a greater buffering power, retaining a greater amount of phosphorus in their colloids, requiring greater fertilization in conditions of low availability. Harter & Barros (2020) observed that calcium supplementation during plant development improved the physiological quality of soybean seeds evaluated by the germination tests, first germination count, electrical conductivity, and cold test. However, it was not evaluated whether this supplementation provided higher concentrations of the nutrient in the produced seeds.

Cultivar DM 66i68 RSF IPRO showed a tendency towards a more pronounced increase in Mg accumulation compared to SYN 15630 IPRO, with a difference observed especially at doses of 150 and 200% of P. Rufino et al. (2018) observed an increase in several variables of initial development, such as dry matter, leaf area and plant height, when soybean seeds of cultivars BRS 243 RR and CD 233 RR were supplemented with calcium, magnesium and silicon via seed coating.

Sulfur (S) concentrations showed contrasting trends among the cultivars evaluated in this study (Table 8). In DM 66i68 RSF IPRO there was no effect of P doses on S concentration, with a general average of 3.44 g of S kg of $seed^{-1}$. For the SYN 15630 IPRO cultivar, a downward trend was observed, ranging from 4.06 to 3.19 g of S kg of $seed^{-1}$ between the doses 0 and 200% of P. The concentrations of S differed between the cultivars for the doses 0%, being superior in SYN 15630 IPRO, and 200% of P the superiority was obtained by the cultivar DM 66i68 RSF IPRO. The results obtained for the SYN 15630 IPRO cultivar are in line with those obtained by Marin et al., (2020), who observed a tendency for a reduction in the S content as the P supply increases. However, the results contrast with those obtained by Singh et al., (2019). Under P-limiting conditions, plants replace membrane phospholipids with sulfolipids and glycolipids (PLAXTON & TRAN, 2021). The results may indicate that the SYN 15630 IPRO cultivar uses this strategy to save P and preserve the integrity of its membranes, while the DM66i68 IPRO cultivar can use the replacement of phospholipids by glycolipids or another strategy.

The micronutrient boron (B) showed a tendency to decrease in concentration with the increase in the dose of added P (table 8). Nutrient concentrations in the seeds ranged from 27.02 to 37.26 mg of B kg of $seed^{-1}$. These values are similar to those observed by Singh et al. (2019) for cv. Spencer that remained between 30 and 42 mg of B kg of $seed^{-1}$. The results obtained by Singh et al. (2019) suggest a linear increase trend for boron content as a function of P supplementation under CO_2 supplemented atmospheres (800 ppm), and a behavior like the quadratic under normal CO_2 concentrations (400 ppm). On the other hand, Marin et al., (2020) did not observe any effect of phosphate fertilizationa on the levels of this micronutrient. The importance of boron in seeds has been associated with the structure of cell walls and membrane integrity (HU & BROWN, 2014; BROWN et al., 2022) especially in the seed coat resulting in greater tolerance to heat stress and delays in harvesting (Bellaloui et al., 2019).

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For the micronutrient cobalt (Co) the concentrations in the seeds obtained in the experiment varied between 0.17 and 0.54 mg of B kg of seed^{-1} (table 8). Dalmolin et al. (2021) observed that foliar fertilization with a commercial product containing Co and Molybdenum (Mo) resulted in an increase in the physiological quality of the seeds produced through the tests of first germination count, standard germination and field emergence.

Copper (Cu), despite its importance as a micronutrient, can lead to toxicity to plants when present in excessive amounts, especially in seeds. The concentrations of Cu in the seeds ranged from 2.28 to 5.69 mg of Cu kg of seed^{-1} (table 8), being much lower than those observed by Marin et al. (2020) and Singh et al., (2019) who observed concentrations between 5 and 12 and between 9 and 14 mg of Cu kg seed^{-1}, approximately.

Rigo (2021) found concentrations ranging from 8.59 to 15.33 mg.kg^{-1} when evaluating 280 lots of 9 different cultivars produced in the Northwest Region of Rio Grande do Sul. Nutrient concentrations tended to decrease with increasing P doses (Table 8) for both cultivars, with this reduction being more intense for the SYN 15630 IPRO cultivar, which differed from DM 66i68 RSF IPRO at doses of 100 and 200% of P. The trend contrasts with the results obtained by Rigo (2021) who observed a positive correlation between Cu contents and the physiological quality of some soybean cultivars in the germination, accelerated aging, viability and vigor tests by the tetrazolium test.

The iron (Fe) levels observed in the seeds of the experiment ranged from 50.21 to 76.48 mg of Fe kg of seed^{-1}. These values are similar to those observed by Marin et al. (2021), between 50 and 60 mg of Fe kg of seed^{-1}. Being quite superior to the results observed by Singh et al. (2019), which were close to 30 mg of Fe kg of seed^{-1}, and at the same time lower than those observed by Rigo (2021), who presented an average value of 97.83 mg of Fe kg of seed^{-1}. The trend towards an increase in iron contents in treatments with greater P availability obtained in this work was also observed by Singh et al. (2019) and Marin et al. (2020). Cultivar SYN 15630 IPRO showed higher Fe contents compared to DM 66i68 RSF IPRO for doses of 100, 150 and 200% of P (table 8).

Manganese (Mn) has an important role in the activity of several enzymes in plants. In soybean seeds produced under application of manganese via foliar fertilization, an increase in the activity of the enzymes alcohol dehydrogenase (ADH), isocitrate lyase (ICL) and a reduction in the enzymes malate dehydrogenase (MDH) and peroxidase (PRX) was observed (Carvalho et al., 2022). The values observed for the seeds of the experiment varied between 85.27 and 203.64 mg of Mn Kg of seed^{-1} (Table 8). These values are much higher than the values observed by Marin et al. (2020), Singh et al. (2019) and Rigo (2021), which varied between 1 and 33 mg of Mn kg of seed^{-1}. However, the observed values are still within the ranges observed by Vargas (2022). These results agree with the results observed by Marin et al. (2015) but contrast with those observed by Singh et al. (2019).

Rigo (2019) observed a positive correlation between the molybdenum (Mo) content in soybean seeds and their physiological quality by the germination, accelerated aging and viability and vigor tests by the tetrazolium test. In table 8 it is possible to observe that the levels observed were low, varying between 0.05 and 2.79 mg of Mo kg of seed^{-1}, compared to those observed by the author, which were between 2.40 and 6.37 mg of Mo kg of seed^{-1}. However, Vargas (2022) observed that the average Mo concentration in 2543 soybean seed lots was 1.95 mg kg^{-1}.

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Zinc (Zn) showed contrasting responses for the evaluated cultivars (Table 8), with a trend towards increased concentrations due to phosphate fertilization for the DM66i68 RSF IPRO cultivar and no response for the SYN 15630 IPRO cultivar. The results for the cultivar DM66i68 RSF IPRO are in agreement with those observed by Marin et al. (2020) and Singh et al. (2019). Additionally, Rigo (2019) observed a positive correlation between zinc concentration in seeds and germination potential, percentage of normal seedlings in the accelerated aging test, viability and vigor in the tetrazolium test.

The determination of physiological quality considers a set of characteristics that can be evaluated in seeds that are directly or indirectly related to seed performance in the field. For the initial development variables, it was possible to test only the seeds produced at doses between 50% and 200% due to the limited amount of seeds produced in the 0% treatment. The results of the analysis of variance demonstrate that there was an interaction between the cultivar and dose factors for the variables electrical conductivity and potassium leaching at 4, 8 and 24 hours, emergence speed index, leaf area and stem dry matter and root ratio and aerial part. For the variable root dry matter, the simple effect of cultivar and dose was observed, while for dry matter of leaves and total dry matter, only the simple effect of dose was observed. For the variables first germination count, germination and seedling emergence in the field, only the simple effect of the cultivar was observed.

Table 9 presents the physiological quality results for the variables where there was a simple cultivar effect. For the variables first count of germination, germination and emergence of plants, the SYN 15630 IPRO cultivar showed superior performance compared to the DM 66i68 RSF IPRO cultivar.

Table 9 - Physiological quality of soybean cultivar seeds tolerant/stable stable (DM66i68 RSF IPRO) and susceptible/responsive (SYN 15630 IPRO) to phosphorus availability.

Cultivar	PCG (%)	G (%)	EC (%)
DM66i68 RSF IPRO	79 b*	89 b	84 b
SYN 15630 IPRO	83 a	94 a	89 a
Average	81	92	87

* Means that do not share a letter, in a same variable, are significantly different by t test at 5% error probability.
Source: Gustavo Zimmer

Seedling emergence was not affected by the amount of available phosphorus. Marin et al. (2020) and Batistella Filho et al. (2023) also did not observe statistical difference for the seedling emergence variable in seeds produced under different doses of phosphorus.

The electrical conductivity at 4, 8 and 24 hours after the beginning of seed imbibition indicated that the SYN 15630 IPRO cultivar showed significantly higher electrical conductivity at the 0% P dose added at all times evaluated, at the 50% P dose for the evaluations at 4 and 8 hours of soaking and the lowest conductivity at the dose of 200% P added at soaking times of 8 and 24 hours. For both cultivars, there was a trend towards a reduction in the values observed at all evaluation times as a response to increased phosphorus fertilization (Figures 2 A, B and C, respectively). The results for potassium leaching showed a similar tendency to electrical conductivity with a reduction in the values observed for the variable in response to increases in added P.

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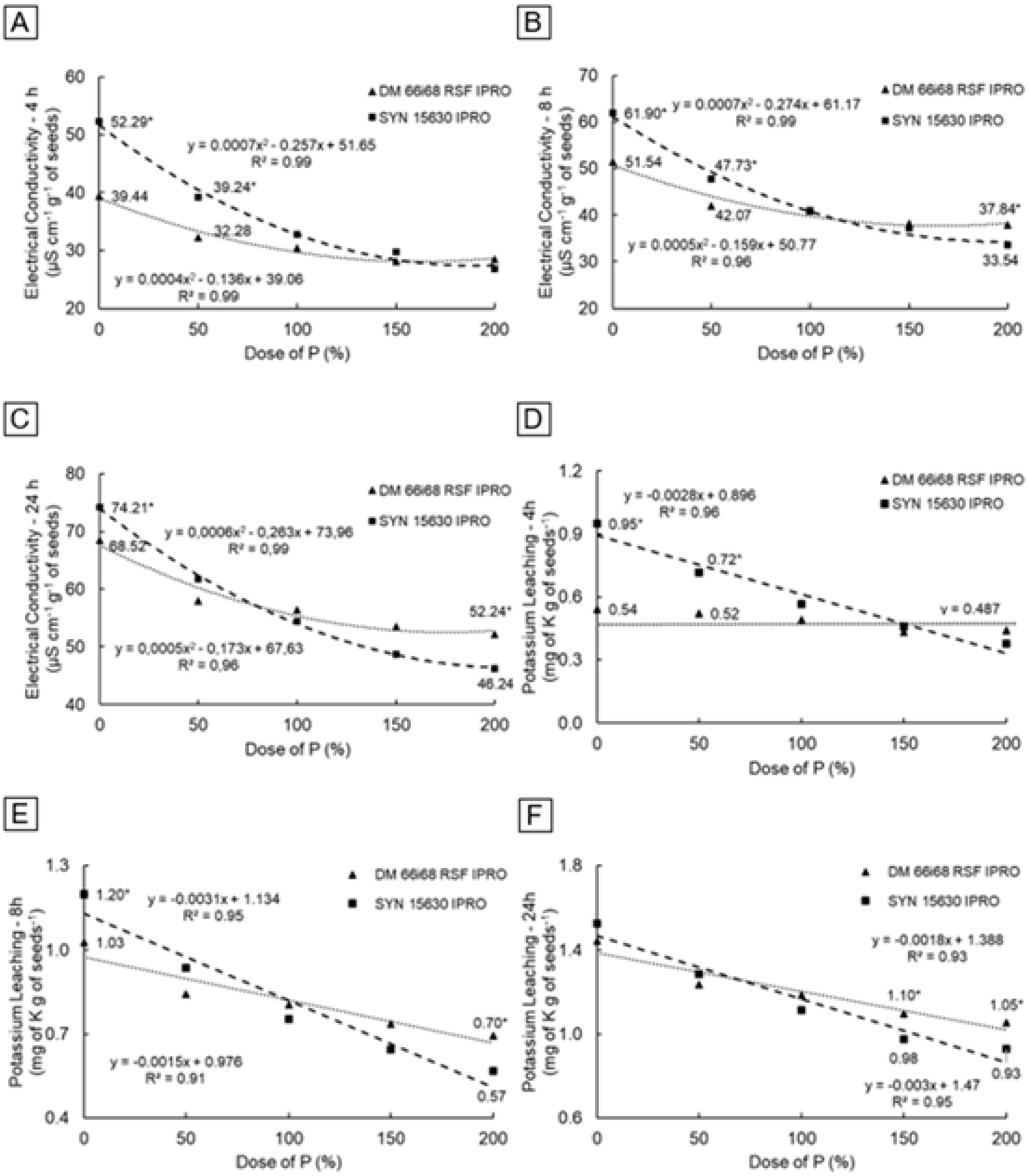
Figure 2 - Physiological quality in seeds of cultivars stable and responsive to phosphorus dose.



Source: Gustavo Zimmer

The SYN 15630 IPRO cultivar showed greater K leaching compared to the DM 66i68 RSF IPRO cultivar at the 0% P dose at 4 and 8 hour evaluation times and at the 50% dose at the 8 hour evaluation time. On the other hand, the cultivar showed lower K leaching at doses of 150% and 200% for the 8hour evaluation and at a dose of 200% P for the 24hour evaluation time. These results contrast the results obtained by Batistella Filho et al. (2023) who did not observe the effect of phosphate fertilization on the electrical conductivity of soybean seeds and adjusted an increasing linear response model for potassium leaching as a function of phosphate fertilization.

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4. FINAL CONSIDERATIONS

The selection of cultivars for levels of tolerance to phosphorus deficiency in experiments that use dry matter production in the early stages of growth has limited potential for application in plant breeding and academic research.

Phosphate fertilization promotes an increase in the number of pods per plant, the number of grains per pod, by reducing the number of pods with 1 grain and increasing the pods with 2 and 3 grains, consequently increasing the number of grains per plant. In addition, it increases the weight of a thousand seeds and the productivity of grains per plant, also observed an improvement in the physiological quality of the seeds determined through the index of speed of emergence and the foliar area of seedlings, the electrical conductivity test, and the leaching test of potassium.

Increasing doses of phosphate fertilizer results in greater accumulation of minerals phosphorus, calcium, magnesium, and iron. For potassium, both an increase and a reduction can occur depending on the cultivar. For sulfur, reduction or absence of response may occur. For zinc, an increase or absence of response may occur depending on the genotype.

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