

## Nitrogen (N) and sulphate (S) fertilization in wheat crop: effect on the vigor of seeds produced

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### Abstract

The objective of this work was to evaluate the influence of nitrogen in association with sulfur applied in the field and the influence of this fertilization on the vigor of wheat seeds produced and later evaluated in the laboratory. Field experiments were carried out in the municipalities of Caibaté-RS and Pelotas-RS using the TBIO Sinuelo cultivar adapted to all the regions of Rio Grande do Sul. The experimental design was a randomized block design with a 2x5x2 trifactorial scheme (cultivation environments x doses of nitrogen x sulfur management) with 4 replicates. The treatments consisted of the combination of two cultivation environments [Caibaté-RS and Pelotas-RS], five nitrogen doses Urea 45%, [0; 22.5; 45; 67.5 and 90 kg ha<sup>-1</sup>] applied at the beginning of tillering and sulfur, Sulfuric 90% sulfur, [0 and 60 kg ha<sup>-1</sup>] applied before sowing, about 7 days. After reaching physiological maturity, the seeds were harvested manually and taken to the seed laboratory. The seeds were dried in a forced ventilation oven at 41°C until the moisture content stabilized at 12% (wet bulb). We evaluated the first germination count (FGC), accelerated aging (AA), field emergence (FE), shoot length (RL) and seedling root length (SL). The seeds produced in Caibaté-RS have greater vigor in relation to Pelotas-RS. Nitrogen fertilization favors the production of seeds with greater vigor, while sulfur can influence the vigor according to the environment in which the plants are cultivated.

**Keywords:** *Triticum aestivum* L., system management, seeds production, quality of seeds.

**Abbreviations:** AA\_ Accelerated aging, FE\_ Field seedling emergence, FGC\_First germination count, N\_Nitrogen, RL\_ Root length, S\_Sulfur, SL\_ Shoot length.

### Introduction

Wheat grains (*Triticum aestivum* L.) are among the main foods used in human and animal feeding, in addition, they have applications in the manufacture of non-food products (Fornasieri Filho, 2008). The Brazilian consumption of this cereal is 9.8 million tons, of which imports 5.4 million tons, corresponding to 55% of the total consumption (Conab, 2017). In the 2016/2017 harvest, the area cultivated with this species in Brazil was 2,118,400 hectares, with a production of over 6.726 tons and a yield of 3175 kg ha<sup>-1</sup>. In this scenario, Paraná and Rio Grande do Sul account for more than 90% of production (Conab, 2017). The increase in yield is dependent, among other factors, on the use of high performance physiological seeds (Abatiet al., 2017).

In Brazil, the average seed utilization rate for the crop is 68%, being below the utilization rate of Rio Grande do Sul, which corresponds to 75% (Abrasem, 2017). The increase in yield is related to the quality of the seed offered to the farmer and, therefore, the management of the seed

production system must be specialized and efficient, in order to obtain the highest seed quality in terms of vigor.

The vigor represents the sum of the physiological attributes of the seed, maintaining relation with the environmental factors, mechanical injury, chemical treatment of seeds and nutrition of the mother plant (Carvalho and Nakagawa, 2012; Meireles et al., 2009; Toledo et al., 2009). The nutrient required in greater quantity by the wheat is the nitrogen, which participates in the formation of amino acids, proteins and the composition of the chlorophyll molecule (Benin et al., 2012; Kutman et al., 2011; Viana and Kihl, 2010).

The requirement for nitrogen is dependent on the genotype, predecessor crop, soil and meteorological conditions (Melero et al., 2013, Nunes et al., 2011) and its efficient use may be adversely affected by sulfur limitation. The Brazilian soil has presented low amounts of sulfur, especially due to the ease of leaching, and in general, the amount required by the plants approximates the nutritional requirement of

phosphorus (Alvares et al., 2007). Some studies studying the application of nitrogen for top dressing showed a beneficial effect on the physiological quality of wheat seeds, increasing the protein concentration in the seeds and the first germination count (Brzezinski et al., 2014; Gul et al., 2012; Warraich et al., 2002).

Nitrogen or sulfur deficiency in plants is manifested at the point of convergence of the assimilatory routes, causing the accumulation or lack of synthesized products (Nikiforova et al., 2006). Sulfur increases the methionine content in cereal proteins and nitrogen can modify the proportions of albumins, globulins, polamines and glutelins (Marschner, 2012; Taiz and Zeiger, 2013). The availability of nutrients to plants can interfere with seed formation in the field, where nitrogen together with sulfur plays important roles in plant metabolism, as they are linked to the composition of amino acids, proteins and chlorophyll. Therefore, critical levels of these nutrients can paralyze the development of the plant, with reflection in the seed produced. Thus, the associated use of nitrogen to sulfur can provide better formed seeds with greater vigor (Mendes et al., 2014). In this context, this work aimed to evaluate the influence of nitrogen in association with sulfur applied in the field and its influence on the vigor of the wheat seeds produced evaluated in the laboratory.

## Results and discussion

The analysis of variance revealed significance for the interaction of nitrogen doses and sulfur management for the shoot length and root length. There was significant interaction for the growing environments x doses of nitrogen for the shoot length and root length. There was interaction for growing environments x doses of nitrogen x sulfur management for the and the first germination count, accelerated aging and field emergence (Table 2).

The interaction between the variation factors revealed the existence of different effects when combined with the variables analyzed, being the response potentiated by referring to the quantitative levels of nitrogen, which can be strongly influenced by the environment. In this way, the environment where the seeds are produced, the nitrogen doses applied for top dressing and the sulfur management modifies the response of the variables. The growing environment factor presents a mean square of higher magnitude in relation to the other sources of variation (Table 2). The experiment had coefficients of variation between 4.4% and 12.1% according to Pimentel Gomes (2009), this results in well-founded and reliable estimates.

The proper functioning of the different cell structures during seed formation, the deposition of reserves with membrane adjustment maintains a close relationship with the vigor of the seeds and this is dependent on the environment in which the seed was produced. Unfavorable environments to the development of seed and matrix, lead to the lower vigor and physiological potential of the seeds (Peske et al., 2012), fact this, evidenced in the present study for the Caibaté-RS growing environment that produced seeds of greater vigor in relation to the growing environment of Pelotas-RS.

Nitrogen fertilization may alter the source-sink relationship due to alteration in crop tillering, modifying the dynamics of fertile tiller emission, as well as seed production (Valerio et al., 2008). According to Thomas (1984), the lack of sulfur can compromise the formation of the embryo and the reserve tissues of the seeds, thus impairing germination and vigor.

Germination did not reveal interaction between the studied factors. However, it is worth mentioning that the germination obtained an average value above 95% being higher than the commercialization standards for this species (Brasil, 2013).

The first germination count was influenced by nitrogen doses, growing environment and sulfur management. For Caibaté - RS, the absence of sulfur resulted in a first germination count adjusted to a quadratic trend with a maximum technical efficiency point in 46 kg ha<sup>-1</sup> with 86% of germinated seedlings (Fig. 2a, Table 5). The use of sulfur showed no response to nitrogen doses (Fig. 2a, Table 5) for the character tested. For Pelotas - RS, when sulfur was used for the first germination count, there was a linear and increasing increment with nitrogen doses 83% for the highest dose (Fig. 2a, Table 5). For the same environment without using sulfur, no significant differences were observed for the first germination count regarding the nitrogen doses (Fig. 2a, Table 5). For the unfolding of the simple effects for the interaction of nitrogen doses x growing environment x sulfur management, revealed that Caibaté - RS with and without sulfur was superior to Pelotas - RS independent of the dose (Table 3). For the environment Pelotas-RS at doses of 0 and 22 kg ha<sup>-1</sup> without sulfur were superior. For the doses of 45 and 67.5 kg ha<sup>-1</sup> when using sulfur, the first germination count was higher and at 90 kg ha<sup>-1</sup> there were no significant differences (Table 3). It was evident for the test of first germination count that the Caibaté - RS environment presents characteristics more propitious for the production of wheat seeds for both cultivars. The increase of the nitrogen doses, together with the sulfur management in specific cases favored the germination of the seeds by the test of the first germination count, and this may be related to the fact that nitrogen and sulfur assimilation are synergistic (Marschner, 2012). It is noteworthy that in Caibaté-RS incident solar radiation was higher than in Pelotas - RS (Fig. 1b, Table 5). The biomass production by the crops is related to the amount of photosynthetically active radiation, intercepted and absorbed by the leaves and the efficiency with which they convert the radiant energy into chemical energy by photosynthetic processes (Heinemann et al., 2006). For in Caibaté - RS the solar radiation was superior and the conversion and deposition of photosynthates in the cells of the endosperm and the development of the embryo structures were more efficient, which increased the percentage of germination of the seedlings through the test of the first count. The environment that the seed is produced is strongly linked with the physiological potential of the seeds, and the environment unfavorable to the development of the seeds and the matrix may impair the germination potential (PESKE et al., 2012). The highest values found in the first germination count are due to the increase in seed vigor expression, being an indication of the greater efficiency in the reorganization of the cell membranes system, hydrolysis and reserve allocation in seedlings (Marcos Filho, 2015). Accelerated aging expressed significant interaction for nitrogen doses x growing environment x sulfur management. For the Caibaté-RS environment with sulfur application, seed vigor decreased with increasing nitrogen doses, which did not occur for Pelotas-RS where the maximum vigor occurred at the dose of 53 kg ha<sup>-1</sup> with 64% (Fig. 2b, Table 5). When comparing the environments for sulfur management, Caibaté-RS showed that the dose of 45 kg ha<sup>-1</sup> presented a difference in

seed vigor due to sulfur applications. For Pelotas-RS the doses of 0 and 22.5 kg ha<sup>-1</sup> of nitrogen increase the vigor of the seeds in the dose of 67.5 kg ha<sup>-1</sup> nitrogen together the presence of sulfur (Table 3).

Nitrogen doses and sulfur management did not have a clear result of their influence on vigor in accelerated aging. In addition, the results suggest that there was a difference in the physiological quality of the seeds, being detected in a test where changes in metabolism occur, including membrane function, nucleic acids, protein synthesis and alterations in metabolism. This test, in addition to providing seed aging, leads to delayed germination, reduced embryo growth and increased susceptibility to environmental stresses, eventually leading to loss of viability (Maia et al., 2007). For Vieira et al. (1995), in wheat, it was verified that the accelerated aging test evidenced that the supply of 120 kg ha<sup>-1</sup> of nitrogen for top dressing in tillering increased seed vigor. Nakagawa et al. (1995), in black oat and by Costa et al. (1983) and Souza et al. (2010) in rice obtained absence of nitrogen effects on seed vigor.

For field emergence evidenced interaction for the nitrogen doses x growing environment x sulfur management. Caibaté-RS without the application of sulfur, showed the maximum efficiency in the dose of 34 kg ha<sup>-1</sup> of nitrogen with 85% of vigor (Fig. 2c, Table 5). In Pelotas-RS the use of sulfur increased the field emergency to 78% in the dose of 90 kg ha<sup>-1</sup> (Fig. 2c, Table 5). The unfolding of the interaction shows that the growing environment Caibaté-RS obtained the most vigorous seeds, the doses of 0; 67.5 and 90 kg ha<sup>-1</sup> in sulfur management did not reveal significance among the tested environments. Among the sulfur managements, Caibaté-RS showed higher seed vigor in the absence of sulfur application at doses of 0; 45 and 67.5 kg ha<sup>-1</sup>, on the other hand, the dose of 90 kg ha<sup>-1</sup> increased the vigor together with the use of sulfur. For Pelotas-RS the doses of 45 and 67.5 kg ha<sup>-1</sup> increased field emergence due to sulfur (Table 3). For Epstein and Bloom (2006), when the two elements are available in sufficient quantity, a better assimilation occurs. Nitrogen may favor protein content and enzymatic activity in seeds, aiding in the greater field emergence (Smiderleet al., 2011) and in the expression of vigor. In a situation of low nitrogen availability, there is a reduction of the protein synthesis in seeds increasing the starch content (Rosa Filho, 2010). The probable explanation for the increased field emergence with dose increase may have been due to the fact that there was an increase in the protein content in the seeds favored by the better use of the resources of the environment, especially the nitrogen fertilization supplied in greater quantity. The increase of the amount of nitrogen increases the area of leaves destined for the capture of solar radiation, and can provide better filling of the seeds (Pimentel et al., 2017; Silva et al., 2003).

For the length of the shoot and length of the root there was interaction between nitrogen doses x sulfur management as well as interaction for nitrogen doses x growing environment. As for the shoot length, it was not significant among the nitrogen doses, but the unfolding interaction showed that only in 0 kg ha<sup>-1</sup> the no sulfur management obtained a better result (Table 4). In relation to the interaction between nitrogen doses and the growing environment, both the Caibaté-RS and Pelotas-RS environments, the shoot lengths were adjusted to a quadratic tendency with a maximum point at the rates of 38 and 45 kg ha<sup>-1</sup> of nitrogen, respectively (Fig. 2d, Table 5).

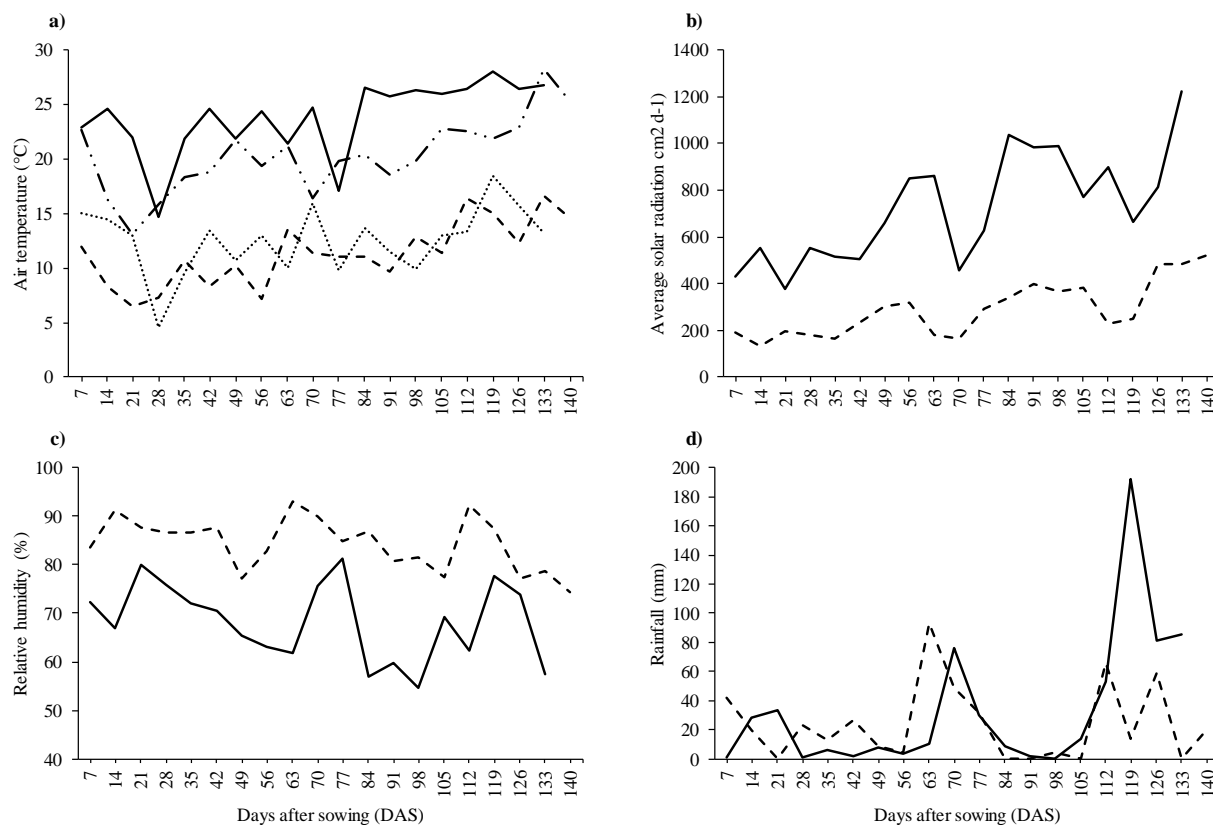
The unfolding of this interaction nitrogen doses x growing environment showed that in the Caibaté-RS environment the seedlings obtained greater shoot length at doses of 0; 22.5, 45 and 67.5 kg ha<sup>-1</sup> and in the dose of 90 kg ha<sup>-1</sup> there were no significant differences between the environments (Table 4). The root length expressed significance for the interaction nitrogen doses x sulfur management, as well as, nitrogen doses x growing environments. The root length when there was no sulfur application was quadratic adjustment with a point of maximum technical efficiency in 41 kg ha<sup>-1</sup>, reaching 63 mm of root length (Fig. 2f, Table 5). Root size increased by 14% up to the maximum technical efficiency dose in relation to seed seedlings originated under 0 kg ha<sup>-1</sup>. When the management was with sulfur application, the root length was not altered by the nitrogen doses (Fig. 2f, Table 5). The unfolding of the interaction shows that in 67.5 kg ha<sup>-1</sup> of nitrogen, the management without sulfur obtained a greater root length, and at the dose of 90 kg ha<sup>-1</sup> the sulfur management reached a longer root length (Table 4). For the Caibaté-RS growing environment, the root length was adjusted to a quadratic tendency with a maximum point of 38 kg ha<sup>-1</sup> and root length reaching 70 mm (Fig. 2e, Table 5). For the Pelotas-RS environment there was a linear and increasing increment of this variable with the increase of the dose, reaching at 90 kg ha<sup>-1</sup> the 52 mm (Fig. 2e, Table 5). By unfolding the simple effects of the significant interaction, the Caibaté environment obtained a longer root length of seedlings at doses of 0; 22.5; 45 and 67.5 kg ha<sup>-1</sup> in relation to the Pelotas environment. At 90 kg ha<sup>-1</sup>, there were no significant differences between the environments (Table 4).

Probably, the greater shoot and root length of the seedlings in the greater doses of nitrogen can be due to the fact that the matrix plant has produced greater leaf area and, with this, it made possible improvement in the chemical composition characteristics in quantity and balance of the seeds with reserve substances, making their germination more vigorous, reaching a larger size of the seedlings (Smiderle et al., 2011). The use of high doses of nitrogen produces the production of growth and developmental phytohormones, which act in processes of cell division and expansion (Marschner, 2012). On the other hand, sulfur plays a role in the constitution of several organic molecules, for example the amino acids cysteine, cystine and methionine, which are found in the composition of most proteins, vitamins biotin and thiamine, coenzyme A, among others (Leclercq, 1992).

Results similar to the present study were found by (Brzezinski et al., 2014) in wheat where the shoot and root length of the seedlings were influenced by the doses of nitrogen. Alves et al. (2017) in wheat research, when analyzing the length of seedlings found that the best response for this test is the dose of 80 kg ha<sup>-1</sup>. In general, the increase in the nitrogen doses provided an improvement in the physiological quality of the seeds. This increase was more visible in the Pelotas-RS growing environment when associated with sulfur, and this result is due, in part, to soil conditions, lower organic matter and more acidic pH, obtaining a more expressive response. The Caibaté-RS growing environment, in general, produced seeds with greater vigor. Among the factors to be considered is the best physical structure of the soil in Caibaté-RS with higher clay content compared to Pelotas-RS, as well as in the higher incident solar radiation.

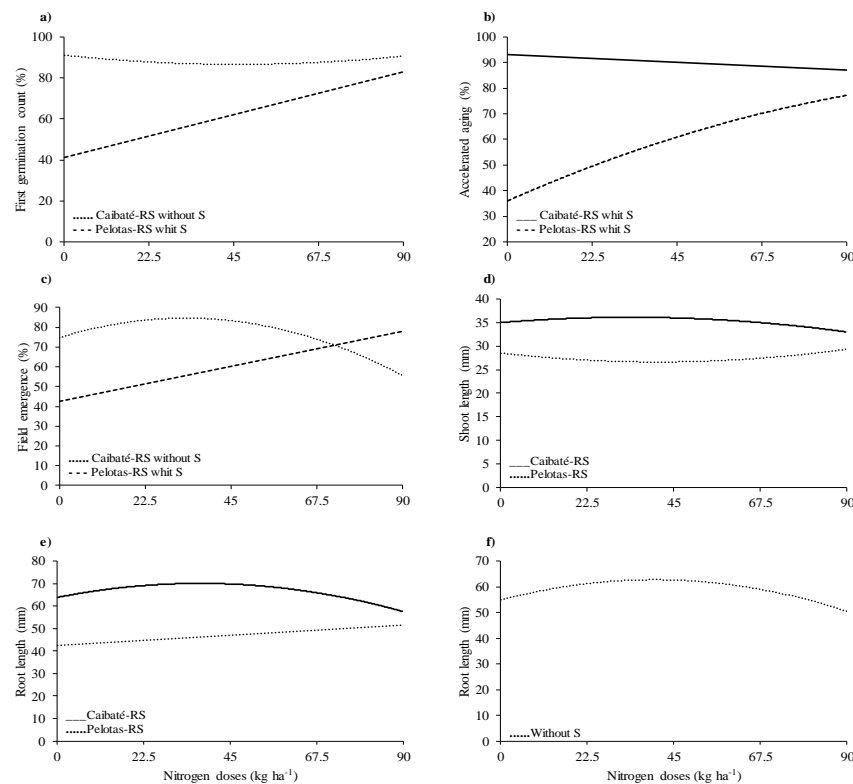
**Table 1.** Chemical and physical analysis of the soils of the experimental areas in the 0,0-0,20 m depth layer.

CAIBATÉ-RS															
pH <sub>(H<sub>2</sub>O)</sub>	-----mg dm <sup>-3</sup> -----								----cmol <sub>c</sub> dm <sup>-3</sup> ----				-----%-----		
	P	K	S	B	Cu	Zn	Mn	Na	Ca	Mg	Al	CTC	V%	M.O	Argila
6.3	8.7	48	12.9	0.0	3.1	1.2	29	6	7.3	3.8	0.0	11.2	84	2.49	43
PELOTAS-RS															
pH <sub>(H<sub>2</sub>O)</sub>	-----mg dm <sup>-3</sup> -----								----cmol <sub>c</sub> dm <sup>-3</sup> ----				-----%-----		
	P	K	S	B	Cu	Zn	Mn	Na	Ca	Mg	Al	CTC	V%	M.O	Argila
5.0	46.0	53	19.8	0.0	1.4	1.8	14.2	50	3.2	0.9	0.8	5.3	45	2.07	19

**Fig 1.** Maximum temperature (—) and minimum air temperature (.....) Caibaté-RS and maximum temperature (-.-.-) and minimum air temperature (---) Pelotas-RS (a), average solar radiation Caibaté-RS (—) and Pelotas-RS (---) (b), relative humidity Caibaté-RS (—) and Pelotas-RS (---) (c) and rainfall Caibaté-RS (—) and Pelotas-RS (---) (d). Source: National Institute of Meteorology (São Luiz Gonzaga-RS) and Agroclimatological Station of Pelotas-RS (University campus Capão do Leão-RS), 2016.**Table 2.** Summary of variance analysis with mean squares for first germination count (FGC), accelerated aging (AA), field emergence (FE), seedling length (SL) and seedling root length (RL) of wheat according to the growing environment, sulfur management and nitrogen doses.

V.F.	D.F.	MEAN SQUARES <sup>(1)</sup>				
		FGC	AA	FE	SL	RL
Environments (E)	1	14580.0*	18757.8*	2553.8*	1082.6*	6559.4*
Sulfur (S)	1	4.0 <sup>ns</sup>	316.0*	140.4 <sup>ns</sup>	10.0 <sup>ns</sup>	163.5*
Doses (D)	4	754.0*	534.0*	1170.5*	2.4 <sup>ns</sup>	183.2*
Block	3	8.6	15.0	47.4	19.0	25.9
E x S	1	4.0 <sup>ns</sup>	6.6 <sup>ns</sup>	806.4*	11.3 <sup>ns</sup>	0.0 <sup>ns</sup>
D x S	4	408.3*	593.1*	391.6*	22.1*	182.6*
E x D	4	508.3*	570.4*	907.8*	43.8*	227.9*
E x D x S	4	422.5*	564.7*	603.4*	3.7 <sup>ns</sup>	71.3 <sup>ns</sup>
Residue	57	15.2	10.3	61.4	7.9	39.2
Average	—	76.0	72.7	64.2	31.9	56.6
CV (%)	—	5.1	4.4	12.1	8.8	11.0

Mean square: \* and <sup>ns</sup> - significant at 5% of probability and not significant, respectively; CV - coefficient of variation.



**Fig 2.** Regressions for interaction of nitrogen doses x growing environment x sulfur management for the variables first germination count (a), accelerated aging (b) and field emergence (c) and for interaction nitrogen doses x growing environment for the variables shoot length (d) and root length (e) and for interaction nitrogen doses x sulfur management for the variable root length (f) (significance level of \* 5% and <sup>ns</sup> not significant).

**Table 3.** Simple effects unfolding of the significant interaction nitrogen doses x growing environment x sulfur management for the variable first germination count (FGC), accelerated aging (AA) and field emergence (FE).

Doses (kg ha <sup>-1</sup> )	FGC (%)				AA (%)				FC (%)			
	Caibaté-RS		Pelotas-RS		Caibaté-RS		Pelotas-RS		Caibaté-RS		Pelotas-RS	
	with S	without S	with S	without S	with S	without S	with S	without S	with S	without S	with S	without S
0	91 Aa*	91 Aa	43 Bb	59 Ba	93 Aa*	89 Aa	38 Bb	53 Ba	47 Ab*	75 Aa	43 Aa	39 Ba
22,5	90 Aa	89 Aa	53 Bb	77 Ba	93 Aa	95 Aa	48 Bb	61 Ba	76 Aa	84 Aa	54 Bb	71 Ba
45,0	86 Aa	84 Aa	54 Ba	47 Bb	87 Aa	68 Ab	54 Ba	51 Ba	65 Ab	80 Aa	52 Ba	41 Bb
67,5	90 Aa	90 Aa	78 Ba	51 Bb	90 Aa	88 Aa	81 Ba	40 Bb	64 Ab	78 Aa	75 Aa	55 Bb
90,0	91 Aa	90 Aa	83 Ba	78 Ba	87 Aa	87 Aa	73 Ba	72 Ba	74 Aa	54 Ab	77 Aa	76 Ba
CV(%)	5.1				4.4				12.1			

\* Means followed by the same capital letter in the line for the growing environment within each sulfur management in each dose of nitrogen and the same lowercase letter for sulfur management within each growing environment at each nitrogen dose do not differ among themselves by the Tukey test at the 5% probability level.

**Table 4.** Simple effects unfolding of the significant interaction nitrogen doses x growing environment and for interaction nitrogen doses x sulfur management for the variables shoot length and root length.

Doses (kg ha <sup>-1</sup> )	SL (mm)		RL (mm)		SL (mm)		RL (mm)	
	Growing environment				Sulfur management			
	Caibaté-RS	Pelotas-RS	Caibaté-RS	Pelotas-RS	with S	without S	with S	without S
0	36.3 A*	28.5 B	65.5 A	42.7 B	30.9 B*	33.9 A	52.0 A	56.2 A
22,5	34.8 A	28.5 B	65.3 A	45.6 B	30.3 A	33.0 A	52.9 A	58.0 A
45,0	36.7 A	27.3 B	75.3 A	49.8 B	33.2 A	30.8 A	59.5 A	65.6 A
67,5	37.7 A	26.7 B	63.8 A	47.3 B	32.7 A	31.7 A	51.6 B	59.5 A
90,0	32.6 A	30.3 A	58.2 A	52.2 A	30.8 A	32.1 A	59.7 A	50.7 B
CV (%)	8.8		11.0		8.8		11.0	

\* Means followed by the same capital letter in the line for growing environment in each dose of nitrogen do not differ among themselves by the Tukey test at the 5% probability level.

**Table 5.** Regression equations for the first germination count variables (FGC), accelerated aging (AA), field emergence (FE), shoot length (SL) and root length (RL).

Variable	Growing environments	Sulfur (S)	Regression equations	R <sup>2</sup>
FGC (%)	Caibaté-RS	With S	$\hat{Y} = ns^{(1)}$	-
FGC (%)	Caibaté-RS	Without S	$\hat{Y} = 91.1 - 0.203x + 0.002x^2$	0.57
FGC (%)	Pelotas-RS	With S	$\hat{Y} = 41.4 + 0.467x$	0.92
FGC (%)	Pelotas-RS	Without S	$\hat{Y} = ns$	-
AA (%)	Caibaté-RS	With S	$\hat{Y} = 93 - 0.07x$	0.68
AA (%)	Caibaté-RS	Without S	$\hat{Y} = ns$	-
AA (%)	Pelotas-RS	With S	$\hat{Y} = 36.6 + 0.641x - 0.002x^2$	0.85
AA (%)	Pelotas-RS	Without S	$\hat{Y} = ns$	-
FC (%)	Caibaté-RS	With S	$\hat{Y} = ns$	-
FC (%)	Caibaté-RS	Without S	$\hat{Y} = 74.6 + 0.603x - 0.009x^2$	0.95
FC (%)	Pelotas-RS	With S	$\hat{Y} = 42.6 + 0.395x$	0.88
FC (%)	Pelotas-RS	Without S	$\hat{Y} = ns$	-
SL (mm)	Caibaté-RS	-	$\hat{Y} = 35.4 + 0.0829x - 0.0011x^2$	0,44
SL (mm)	Pelotas-RS	-	$\hat{Y} = 29.0 - 0.0892x + 0.0010x^2$	0.61
RL (mm)	Caibaté-RS	-	$\hat{Y} = 64.2 + 0.3393x - 0.0045x^2$	0.66
RL (mm)	Pelotas-RS	-	$\hat{Y} = 43.4 + 0.0913x$	0.78
RL (mm)	-	With S	$\hat{Y} = ns$	-
RL (mm)	-	Without S	$\hat{Y} = 54.9 + 0.4006x - 0.0049x^2$	0.84

<sup>(1)</sup> Not significant at 5% probability by t test.

## Materials and methods

### Study site

The experiment was conducted in the field in the 2016 agricultural crop in two environments of Rio Grande do Sul, located in Caibaté-RS at the geographic coordinates of 28°17'16" south latitude and 54°38'16" west longitude and average altitude of 286 meters, and Pelotas-RS at the coordinates of 31° 52'00" south latitude and 52° 21'00" west longitude and average altitude of 13 meters. The climate for both environments is classified as subtropical humid Cfa type according to Köppen.

### Soil classification

The soil of Caibaté-RS is characterized as Typical Red Dystrophic Latosol (EMBRAPA, 2013), while in Pelotas-RS the soil is characterized as Solodic Eutrophic Haplic Planosol, belonging to the Pelotas mapping unit (STRECK et al., 2008). The basic fertilization was carried out at the time of sowing, with 350 kg ha<sup>-1</sup> in Caibaté-RS and 500 kg ha<sup>-1</sup> in Pelotas-RS of NPK fertilizer 11-30-20 according to soil analysis (Table 1) and recommendations of the Committee on Soil Chemistry and Fertility (CQFS-RS / SC, 2004).

### Climate conditions

The climatic data of air temperature, average solar radiation, relative humidity and rainfall were collected in meteorological station, being the averages of maximum and minimum temperature of the air, maximum solar radiation, relative humidity of the maximum and minimum air and precipitation pluviométrica accumulated in the experiment period for Caibaté-RS at 28°C and 9°C, 1224 cal cm<sup>2</sup> d<sup>-1</sup>, 80% and 54% and 635 mm, respectively (Fig. 1). For Pelotas-RS, mean maximum and minimum air temperature, mean maximum solar radiation, maximum and minimum air relative humidity and accumulated rainfall during the experiment period were 28°C and 6°C, 518 cal cm<sup>2</sup> d<sup>-1</sup>, 92% and 74% and 475 mm, respectively (Fig. 1).

### Management of fertilization and description of plant material

In both experimental environments, top dressing nitrogen fertilization was performed at the beginning of tillering, using urea source (45% of nitrogen), using doses of 0; 22.5; 45; 67.5 and 90 kg ha<sup>-1</sup> of nitrogen. Before the sowing, sulfur fertilization was carried out, using the active principle with 90% sulfur in the dose of 60 kg ha<sup>-1</sup>, according to the recommendation.

The cultivar 'TBIO Sinuelo' were used, recommended for all the growing wheat regions of the Southern Region of Brazil. The seeds were treated with fungicide (50% a.i. Carbendazim + 15% a.i. Difeconazole), insecticide (21% a.i. Thiametoxan + 3.75% ia Lambda-cyhalothrin), using 80 mL per 40 kg of seeds and mineral supplement (13% of total nitrogen, 5% of potassium oxide (K<sub>2</sub>O), humic and fulvic acids, glycine betaine and zeatin) at a dose of 50 mL per 40 kg of seeds. The sowing was performed on 06/24/2016 (Caibaté-RS) and 07/01/2016 (Pelotas-RS), using the seeding density of 350 seeds per square meter for each cultivar in both growing environments. Each experimental unit

consisted of 5 lines spaced at 0.17 meters with 3 meters in length.

### Experimental design

The experimental design was a randomized block arranged in a 2 x 5 x 2 factorial scheme (growing environments x doses of nitrogen x sulfur managements) for both cultivars. The treatments consisted of a combination of two growing environments [Caibaté-RS and Pelotas-RS], five nitrogen doses (Urea 45%), [0; (without application); 22.5; 45; 67.5 and 90 kg ha<sup>-1</sup>] and sulfur (Sulfulgran 90% sulfur), [0; (without application); 60 kg ha<sup>-1</sup>] being arranged in four replicates. The weed control, pest insects and diseases were carried out in accordance with the technical recommendations for the wheat crop.

### Harvesting seeds

After the physiological maturity, the seeds were harvested manually with moisture content between 18 and 20%. For the harvest, the three central lines of the plot with two meters long were considered useful area, scoring 0.5 meters at each end of the experimental unit. The seeds were submitted to forced ventilation drying at 41°C air temperature until the moisture content stabilized at 12% (wet bulb). The processing was carried out according to recommendations for the seeds of this species (Silva et al., 2017), being the seeds stored in a cold and dry chamber, with control of temperature and relative humidity of the air until the tests were carried out.

### Measured variables

For the evaluation of the response of plants to the nitrogen doses in association with sulfur regarding the physiological attributes of wheat seeds, the following attributes were analyzed in the Laboratory of Seed Analysis of the Postgraduate Program in Science and Technology of Seeds:

*First germination count (FGC)*: obtained by means of four samples with four subsamples of 50 seeds for each treatment, the seeds being arranged to germinate between three *germitest* germination paper sheets, moistened 2.5 times the mass of the dry substrate. The rolls were kept in a BOD type germination chamber at a temperature of 20°C. The counting was performed four days after sowing and the results were expressed as percentage of normal seedlings (Brasil, 2009).

*Accelerated aging (AA)*: for this purpose, the seeds were distributed on metallic screens fixed inside plastic boxes ("gerbox"), containing 40 mL of saturated saline solution (Pedroso et al., 2010). The saturated saline solution was composed of 11 grams of NaCl per 100 mL of water and the plastic boxes containing the seeds were kept in BOD at 43°C for 48 h (Lima et al., 2006). After the time, the seeds were arranged to germinate under the same conditions of the germination test, the germination count being four days after sowing and the results expressed as percentage of normal seedlings (Brasil, 2009).

*Field seedling emergence (FE)*: evaluated from the sowing of four replicates of 100 seeds per treatment, in beds containing soil. The evaluation was performed at 21 days after sowing, determining the percentage of seedling emergence (Nakagawa, 1994).

*Shoot length and root length (SL and RL)*: for seedling length evaluation the seeds were submitted to procedures adapted from AOSA (1983) and described by Nakagawa (1999). Four subsamples of 20 seeds per replicate were used. The seeds were arranged in the upper third in the longitudinal direction of the paper. The rolls were packed in plastic bags positioned vertically in a BOD-type germinator regulated at 20°C for eight days. Afterwards, the normal seedlings (shoot length and root length) were measured using a millimeter ruler. The mean results were expressed in millimeters.

### Statistical analyses

The data were submitted to analysis of variance at 5% of probability, where their assumptions were verified. The diagnosis of the interaction between growing environments x doses of nitrogen x sulfur management at 5% of probability was performed, the interaction when significant was dismantled to the qualitative variation factors (growing environments and sulfur management) to the simple effects, on the other hand, the quantitative levels (nitrogen doses) were submitted to polynomial regression, where the significant degree of polynomial for each level of qualitative treatment was verified by t test at 5% of probability. The characters that did not show interaction were submitted to the dismemberment of the main effects through the complementary analyzes by Tukey at 5% of probability for qualitative variation factors. For the quantitative effects, the general polynomial regression was adjusted with the degree of the polynomial to 5% by the t test.

### Conclusions

The seeds produced in Caibaté - RS are more vigorous compared to those produced in Pelotas - RS.

Nitrogen favors the production of seeds with greater vigor, whereas, sulfur can influence the vigor according to the growing environment.

In Pelotas - RS for the dose of 90 kg ha<sup>-1</sup> of nitrogen in association with sulfur when applied results in higher vigor seeds.

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### References

- Abati J, Brzezinski CR, FOLONI JSS, Zucareli C, Bassoi MC, Henning FA (2017) Seedling emergence and yield performance of wheat cultivars depending on seed vigor and sowing density. *J Seed Sci.* 39(1): 058-065.
- Alvarez RCF, Crusciol CA, Ocheuze PC, Rodrigues JD, Alvarez ACC (2007) Influencia do etil-trinexapac no acúmulo, na distribuição de nitrogênio (<sup>15</sup>N) e na massa de grãos de arroz de terras altas. *Revista Brasileira de Ciências do Solo.* 31: 1487-1496.
- Alves SRP, Francisco ALO, Carvalho TC (2017) Azospirillum brasilense e nitrogênio: atuação no potencial fisiológico de sementes de trigo. *Applied Research and Agrotechnology.* 10(2): 43-51.
- Associação Brasileira De Sementes e Mudas (2015). *Yearbook Available at*: <[http://www.abrasem.com.br/wp-content/uploads/2013/09/Anuario\\_ABRASEM\\_2015\\_2.pdf](http://www.abrasem.com.br/wp-content/uploads/2013/09/Anuario_ABRASEM_2015_2.pdf)>. AOSA (1983). *Seed vigor testing hand book.* East Lansing: AOSA.
- Benin G, Bornhofen E, Beche E, Pagliosa ES, Silva CL, Pinnow C (2012) Agronomic performance of wheat cultivars in response to nitrogen fertilization levels. *Acta Scientiarum Agronomy.* 34: 275-283.
- Brasil - Ministério Da Agricultura E Da Reforma Agrária (2009) *Regras para análise de sementes.* Brasília: Secretaria de Defesa Agropecuária. Brasília: Mapa/ACS.
- Brasil - Instrução Normativa nº 45, de 17 de setembro de 2013 (2013) *Diário Oficial da República Federativa do Brasil, Ministério da Agricultura, Pecuária e Abastecimento, Brasília, DF.*
- Brzezinski CR, Zucareli C, Henning FA, Abati J, Prando AM, Henning AA (2014) Nitrogênio e inoculação com Azospirillum na qualidade fisiológica e sanitária de sementes de trigo. *Revista de Ciências Agrárias.* 57(3): 257-265.
- Carvalho NM, Nakagawa J (2012) *Sementes: Ciência, Tecnologia e Produção.* Jaboticabal: FUNEP.
- Cqfs (2004) *Manual de adubação e calagem para os Estados do Rio Grande do Sul e Santa Catarina, 10ª ed, Sociedade Brasileira de Ciência do Solo. Comissão de Química e Fertilidade do Solo, Porto Alegre.*
- Conab - Companhia Nacional de Abastecimento (2017). *Acompanhamento da safra Brasileira: grãos, sétimo levantamento abril 2017/ Companhia Nacional de Abastecimento, Brasília: Conab 2017.* [http://www.conab.gov.br/olalaCMS/uploads/arquivos/16\\_04\\_07\\_10\\_39\\_11\\_boletim\\_graos\\_abril\\_2017.pdf](http://www.conab.gov.br/olalaCMS/uploads/arquivos/16_04_07_10_39_11_boletim_graos_abril_2017.pdf).
- Costa NP, Gomes AS, Peske ST, Popinigis F, Zonta EP (1983) Influência da adubação nitrogenada sobre o vigor e conteúdo de proteína de sementes de quatro cultivares de arroz irrigado. *Revista Brasileira de Sementes.* 5(1): 31-41.
- Empresa Brasileira De Pesquisa Agropecuária – Embrapa (2003). *Sistema Brasileiro de Classificação de Solos.* 3 ed. Rio de Janeiro: Embrapa Solos.
- Epstein E, Bloom A (2006) *Nutrição mineral de plantas: princípios e perspectivas.* 2.ed. Londrina: Editora Planta.
- Fornasieri Filho, D (2008). *Manual da cultura do trigo.* Jaboticabal: Funep.
- Heinemann AB, Stone LF, Didonet AD, Trindade MG, Soares BB, Moreira JAA, Canovas AD (2006) Eficiência de uso da radiação solar na produtividade de trigo decorrente da adubação nitrogenada. *Revista Brasileira de Engenharia Agrícola e Ambiental.* 10(2): 352-356.
- Kutman UB, Yildiz B, Cakmak I (2011) Effect of nitrogen on uptake, remobilization and partition in gof zinc and iron through out the development of durum wheat. *Plant and Soil.* 342(1-2): 149-164.
- Larbier M, Leclercq B (1992) *Nutrition and Feeding of poultry.* Nottingham: Nottingham University.
- Lima TC, Medina PF, Fanan P (2006) Avaliação do potencial fisiológico de sementes de trigo pelo teste de envelhecimento acelerado. *Revista Brasileira de Sementes.* 57(2): 106-113.
- Maia AR, Lopes JC, Teixeira CO (2007) Efeito do envelhecimento acelerado na avaliação da qualidade fisiológica de sementes de trigo. *Ciência e Agrotecnologia.* 31(3): 678-684.
- Marcos-Filho J (2015) *Fisiologia de sementes de plantas cultivadas.* 2. ed. Londrina: ABRATES.
- Marschner H (2012) *Mineral nutrition of higher plants.* 3. ed. London: Elsevier.



- Meireles RC, Silva RF, Araújo EF, Reis LS, Lyra GB, Marinho AB (2009) Influência do nitrogênio e das lâminas de irrigação na qualidade fisiológica das sementes de mamoeiro. *Revista Brasileira de Sementes*. 31: 216-221.
- Mendes MC, Walter ALB, Junior OP, Rizzardi DA, Schlosser J, Szeuczuk K (2014) Dose de nitrogênio associado a enxofre elementar em cobertura na cultura do milho em plantio direto. *Revista Brasileira de Milho e Sorgo*. 13(1): 96-106.
- Nakagawa J (1994) Teste de vigor baseados na avaliação de plântulas. In: Vieira, RD, Carvalho, NM. Testes de vigor em sementes. Jaboticabal: FUNEP
- Nakagawa J (1999) Testes de vigor baseado no desempenho de plântulas. In: Kryzanowski, F. C.; Vieira, R. D.; França Neto, J. B. (Ed.). Vigor de sementes: conceitos e testes. Londrina: Abrates.
- Nakagawa J, Cavariani C, Machado JR (1995) Efeitos da dose de aplicação de N na produção e qualidade de sementes de aveia preta. *Científica*. 23(1): 31-43.
- Nikiforova VJ, Bielecka M, Gakiere B, Krueger S, Rinder J, Kempa S, Morcuende R, Scheible WR, Hesse H, Hoefgen R (2006) Effect of sulfúfur availability on the integrity of amino acid biosynthesis in plants. *Amino Acids*. 30: 173-183.
- Nunes AS, Souza LCF, Vitorino ACT, Mota LHS (2011) Adubos verdes e doses de nitrogênio em cobertura na cultura do trigo sob plantio direto. *Semina: Ciências Agrárias*. 32(4): 1375-1384.
- Pedroso DC, Tunes LM, Barbieri AP, Barros ACSA, Muniz MFB, Menezes VO (2010) Envelhecimento acelerado em sementes de trigo. *Ciência Rural*. 40(11): 2389-2392.
- Peske ST, Villela FA, Meneguello GE (2012) Sementes: Fundamentos Científicos e Tecnológicos, 3ed.
- Pimentel Gomes F (2009). Curso de estatística experimental. 15.ed. Piracicaba: FEALQ.
- Pimentel JR, Monteiro MA, Aumonde TZ, Pedó T (2017) Nutrição de plantas, vigor de sementes e estresses abióticos. In: Aumonde, T. Z; Pedó, T.; Martinazzo, E. G.; Villela, F. A. Estresses ambientais e a produção de sementes: Ciência e Aplicação. 1ª ed. Pelotas: Cópias Santa Cruz.
- Rosa Filho O (2010) Introdução ao manejo para qualidade industrial em trigo. Informativo Técnico Biotrigo, Passo Fundo.
- Ilva RS, Bassoi MC, Foloni JSS (2017) Informações técnicas para trigo e triticale – safra 2017. In: X Reunião da Comissão Brasileira de Pesquisa de Trigo e Triticale, Brasília, DF: Embrapa.
- Silva AS, Carvalho FIF, Nedel JL, Cruz PJ, Peske ST, Simioni D, Cargnin A (2003) Enchimento de sementes em linhas quase-isogênicas de trigo com presença e ausência do caráter “stay-green”. *Pesquisa Agropecuária Brasileira*. 38(5): 613-618.
- Smiderle OJ, Chang MT, Ferreira GB, Cordeiro ACC (2011) Qualidade de sementes de arroz BRS jaçanã em função de aplicações de nitrogênio. *Rev. Acad., Ciênc. Agrár. Ambient*. 9(1): 79-86.
- Sotério PW, Pedrollo MC, Andriotti JL (2005) Mapa de isoietas do Rio Grande do Sul. Porto Alegre.
- Souza LCD, Sá ME, Martins HSD, Abrantes FL, Silva MP, Arruda N (2010) Produtividade e qualidade de sementes de arroz em resposta a doses de calcário e nitrogênio. *Revista Trópica*. 4(2): 27-35.
- Taiz L, Zeiger E (2013) Fisiologia vegetal. 5. ed. Porto Alegre.
- Thomas P (1984) Canola growers manual: growwith canola. Canola Council of Canada, Winnipeg.
- Toledo MZ, Fonseca NR, Cesar ML, Soratto RP, Cavariani C, Crusciol CAC (2009) Qualidade fisiológica e armazenamento de sementes de feijão em função da aplicação tardia de nitrogênio em cobertura. *Pesquisa Agropecuária Tropical*. 39: 124-133.
- Valério IP, Carvalho FIF, Oliveira AC, Machado AA, Benin G, Scheeren PL, Souza VQ, Hartwig I (2008) Desenvolvimento de afilhos e componentes do rendimento em genótipos de trigo sob diferentes densidades de semeadura. *Pesquisa Agropecuária Brasileira*. 43(3): 319-326.
- Viana EM, Kiehl JC (2010) Doses de nitrogênio e potássio no crescimento do trigo. *Bragantia*. 69: 975-982.
- Vieira RD, Fornasieri Filho D, Bergamaschi CM (1995) Efeito de doses e de épocas de aplicação de nitrogênio em cobertura na produção e na qualidade fisiológica de sementes de trigo. *Científica*. 23(2): 257-254.
- Warraich EA, Basra SMA, Ahmad N, Ahmed R, Aftab M (2002) Effect of nitrogen on grain quality and vigour in wheat (*Triticum aestivum* L.). *International Journal of Agriculture and Biology*. 4 (4): 517-520.