

# Nitrogen and Sulfur Association: Effects on Yield Components and Physical Attributes of Wheat Seeds

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## Abstract

The aimed to evaluate the effect of different nitrogen doses in association with sulfur fertilization on yield components and seeds physic-quality attributes. The experimental design was randomized blocks, arranged in a  $3 \times 5 \times 2$  factorial design (cultivar  $\times$  nitrogen doses  $\times$  sulfur management), with four replications. The analysis of variance revealed that wheat cultivars  $\times$  sulfur management interaction was significant for height insert of main spike. Interaction was verified between cultivars  $\times$  nitrogen doses for number of tillers per plant and number of spikes per  $m^2$ . The nitrogen doses  $\times$  sulfur management interaction was significant for number of tillers per plant. Interaction between cultivars  $\times$  nitrogen doses were observed for number of spicules per spikes and number of seeds per spike. Nitrogen fertilization has different effects on wheat cultivars. The cultivar 'TBIO Sinuelo' presents greater seeds yield with improvement of nitrogen fertilization doses. High nitrogen doses provide increasing of wheat seeds yield. Nitrogen doses influence differently on yield components of wheat. Number of tillers per plant is correlated with seeds yield. Physical attributes of wheat seeds are not influenced by nitrogen doses increasing. Sulfur fertilization promotes tillering and increment of number of spicules per spike. The cultivar 'TBIO Sinuelo' presents higher weight per 1000 seeds, followed by 'Jadeite 11', and 'TBIO Sintonia'.

**Keywords:** *Triticum aestivum* L., agronomic ideotype, seed yield

## 1. Introduction

Wheat (*Triticum aestivum* L.), from Poaceae family, is one of the main winter cereals cultivated worldwide. Due to grains great versatility, good nutritional characteristics, and easy storage, this species has become a staple food for the population (Sleper & Poehlman, 2006; Carvalho et al., 2017).

Brazilian wheat consumption is 9.8 million tons, and 5.4 million tons out of it are imported, which represents 55% of total consumption. During the 2016/2017 crop year, wheat Brazilian field's production was 2,118,400 hectares, producing 6,726,800 ton of grains, and yield of  $3175 \text{ kg ha}^{-1}$ . The states of Paraná, Rio Grande do Sul, and Santa Catarina has accounted 91% of total production, out of which Rio Grande do Sul produced 776,900 ha, with  $3,214 \text{ kg ha}^{-1}$  of yield, totaling 2,497,000 ton of wheat (CONAB, 2018).

Wheat plants are very demanding for good balanced fertilization. Excess of fertilizer, especially nitrogen, may lead to plants lodging. Nitrogen excess during grain filling reduces yield, hectoliter weight and hamper harvesting (Zagonel & Fernandes, 2007; Kelh et al., 2016). Otherwise, low nitrogen doses may restrict yield (Teixeira Filho et al., 2010; Demari et al., 2018).

Fertilization is essential to obtain high yields (Ferrari et al., 2016). The nutrients absorption rate is controlled by external concentrations, and by plant demand for growing and developing. Nitrogen and potassium are the main demanded nutrients by wheat plants. Nitrogen is needed in higher amounts, since is fundamental to synthesize amino acids and proteins, besides been a chlorophyll molecules compound, which means that it is substantial for plant growing and development, and for seeds production (Fuertes-Mendizabal et al., 2010; Prando et al., 2012; Taiz & Zeiger, 2013). In its turn, potassium is substantial for enzymes activation and cellular turgidity

maintenance. The phosphorus is the fertilizer applied in highest amount in corrective and maintenance fertilization, due to the low amount of this nutrient in soil, and low viability to plants (Rajj, 2011).

The nutrient sulfur is part of amino acids and proteins composition. Also, it is important for roots development (Dias, 2012; Lavres Júnior et al., 2008; Stipp & Casarin, 2010). Sulfur restriction may result in yield reduction, mainly because it limits the efficient nitrogen use (Sahota, 2006). Recently released wheat cultivars are responsive to nitrogen availability, so than plants can reach high seeds yield and quality, which makes sulfur a fundamental nutrient in this process (Rodrigues & Teixeira, 2010).

Seeds yield can be measured by several components, like the number of spikes per plant, spicules per spike, seeds per spike/spicules, and seeds weight. All these characters are related to genetic and environmental factors, besides soil fertilization (Cruz et al., 2003; Fioreze, 2011; Szarecki et al., 2017). In this sense, individual analysis of yield components can be associated with morphological parameters, such as plant grow and development (Espindula et al., 2014; Carvalho et al., 2017).

This study aimed to evaluate the effect of different nitrogen doses in association with sulfur fertilization on yield components and seeds physic-quality attributes.

## 2. Material and Methods

The trial was carried out in 2016, in a seeds production field at Caibaté-RS, Brazil. The latitude is 28°17'16" S, longitude 54°38'16" W, and altitude of 286 meters above sea level. The climate is characterized by Köppen as "Cfa", subtropical with well distribute rainfall throughout the year. The year average temperature is about 20 °C to 22 °C; the winter is defined and average rainfall of 1250 mm to 2000 mm. Values of air temperature, solar radiation, relative humidity and rainfall were recorded during experimental time (Figure 1).

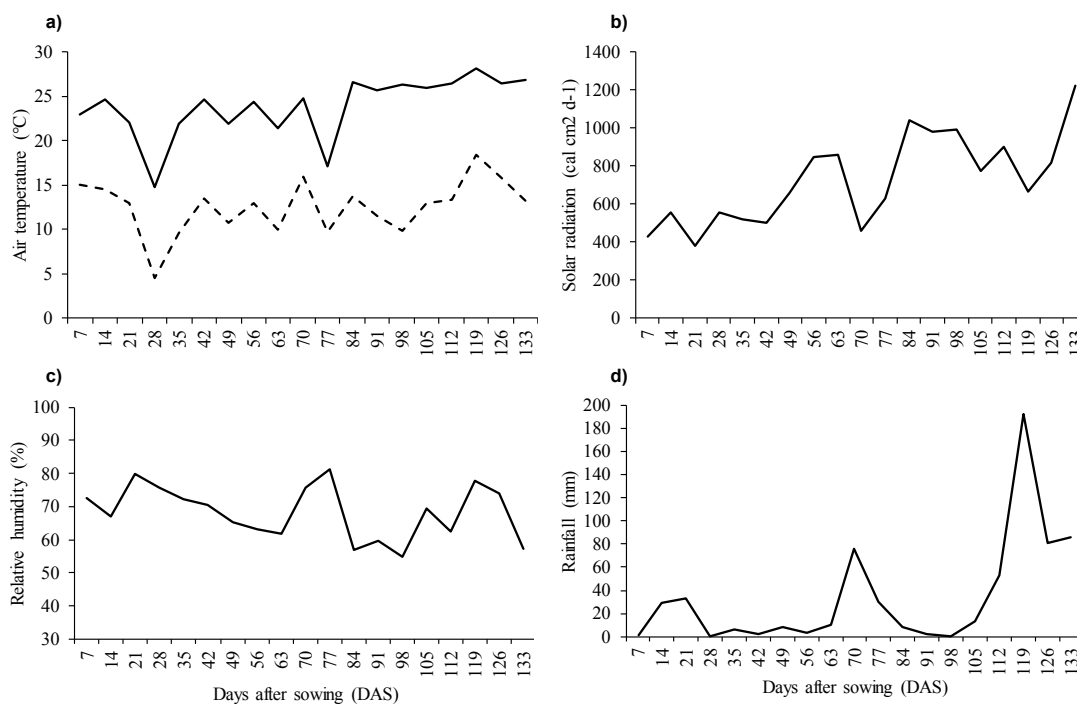


Figure 1. Maximum air temperature (—) and minimum air temperature (- - -) (a), solar radiation (b), relative humidity (c) e rainfall (d). Source: National Institute of Meteorology. Caibaté, 2016

The soil is classified as Typic Dystrophic Red Latossol (EMBRAPA, 2013), and chemical and physical characteristics are: pH (H<sub>2</sub>O): 6.3; P: 8.7 mg dm<sup>-3</sup>; K: 48 mg dm<sup>-3</sup>; S: 12.9 mg dm<sup>-3</sup>; Ca: 7.3 cmol<sub>c</sub> dm<sup>-3</sup>; Mg: 3.8 cmol<sub>c</sub> dm<sup>-3</sup>; Al: 0.0 cmol<sub>c</sub> dm<sup>-3</sup>; B: 0.0 mg dm<sup>-3</sup>; Cu: 3.1 mg dm<sup>-3</sup>; Zn: 1.2 mg dm<sup>-3</sup>; Mn: 29 mg dm<sup>-3</sup>; Na: 6 mg dm<sup>-3</sup> CTC: 11.2 cmol<sub>c</sub> dm<sup>-3</sup>; 84% of base saturation; 2.49% of organic matter; and 43% of clay.

Corrective fertilization was performed at sowing through the addition of 350 kg ha<sup>-1</sup> of NPK 11-30-20 fertilizer to the soil, according to recommendations from the Commission of Soil Chemistry and Fertility, based on the results of soil analysis (CQFS-RS/SC, 2004). Nitrogen top dressing was made at tillering, 45 days after sowing,

the fertilizer urea (45% of nitrogen) was spread on doses of 0.00; 22.5; 45.0; 67.5; and 90.0 kg ha<sup>-1</sup> of nitrogen. Seven days before sowing, sulfur fertilization was performed in the dose of 60 kg ha<sup>-1</sup>, using a commercial product containing 90% of sulfur.

Wheat seeds were sowed at June 24<sup>th</sup>, 2016, in a 350 seeds m<sup>-2</sup> density. Each experimental unit was composed by five rows with 3m of length, in 0.17m wide. The experimental design was randomized blocks, arranged in a 3 × 5 × 2 factorial design (cultivar × nitrogen doses × sulfur management), with four replications. The weeds, insects and pests control was made according to recommendations for wheat crop.

Three wheat cultivars were tested in this study, 'TBIO Sintonia', 'TBIO Sinuelo' and 'Jadeíte 11', all adapted for Rio Grande do Sul and Parana wheat producer regions. Seeds were industrially treated with fungicide (50% i.a Carbendazim + 15% i.a of Difeconazol), insecticide (21% i.a of Thiametoxan + 3.75% i.a of Lambda-cialotrina), using 80mL per 40Kg of seeds, and mineral supplement (13% of total nitrogen, 5% of potassium oxide (K<sub>2</sub>O), humic and fulvic acids, glycine-betayne and zeatyne) in the dose of 50 mL per 40 Kg of seeds.

Seeds were handily harvested at October 29<sup>th</sup>, 2016, when they reached 18 to 20% of humidity. The three central rows in each plot were considered as useful area, and 0.5m from each edge were discarded. Harvested seeds were dried in a forced ventilation at 41 °C of air temperature until consistence of 12% of humidity (wet bulb). Seeds processing was performed according to recommendations for wheat seeds (Silva et al., 2017), following to cold and dry storage, with humidity and temperature control.

To evaluate the effect of nitrogen doses in association with sulfur management on yield, yield components, and physic quality of wheat cultivars, the following parameters were assessed:

*Plant height*: ten plants were selected at random from each experimental unit, and measured with a ruler, as the distance from ground level to the spikes tips, means were taken and the results were expressed in cm.

*Number of tillers*: ten plants were selected at random from each experimental unit, their number of tillers were counted and means were taken, results expressed in tiller plant<sup>-1</sup>.

*Number of spicules per main spike*: the number of spicules from ten spikes were counted and means were taken, results expressed in spicules spike<sup>-1</sup>.

*Height insert of main spike*: ten plants were selected at random from each experimental unit, the height insert of main spike was assessed from ground with a ruler. Means were taken, and results were expressed in cm.

*Number of seeds per spike*: ten plants were randomly selected from each experimental unit, the seeds from all the spikes were counted and the results were expressed in seeds spike<sup>-1</sup>.

*Number of spikes per m<sup>2</sup>*: an area of 1 m<sup>2</sup> was selected at random in each experimental unit to count the number of spikes, the results were expressed in spikes m<sup>-2</sup>.

*Weight per 1000 seeds*: eight samples of 100 seeds were selected at random from experimental unit, seeds were counted and weighted, the results were expressed in g (Brasil, 2009).

*Hectoliter weight*: seeds from each experimental unit were weighted in a hectoliter scale with one quarter of liter capacity. Results were expressed in kg hL<sup>-1</sup> (Brasil, 2009).

*Seeds yield*: the weight of seeds from each experimental unit (corrected for 12% humidity) were recorded, in kg. Results expressed in kg ha<sup>-1</sup>.

Data recorded were analyzed by the analysis of variance, using F test at 5% of probability. In the presence of interaction between cultivars × nitrogen doses × sulfur management, the qualitative characters (cultivars and sulfur management) were sliced into simple effects. Quantitative characters were analyzed by polynomial regression at 5% of probability, and the higher significant polynomial degree was determined for each level of the quantitative treatments. Qualitative characters that did not present significant interaction were sliced into principal effects for each factor by Tukey test, at 5% of probability. Not significant quantitative characters were analyzed by linear regression with adjust of the higher degree of polynomial at 5% of probability by t test.

### 3. Results and Discussion

The averages of maximum and minimum air temperature, maximum solar radiation, maximum and minimum relative humidity and accumulated rainfall during the growing season were 28 °C and 10 °C; 1224 cal cm<sup>2</sup> d<sup>-1</sup>; 80% and 54%; and 635 mm, respectively (Figure 1).

The analysis of variance revealed that wheat cultivars × sulfur management interaction was significant for height insert of main spike. Interaction was verified between cultivars × nitrogen doses for number of tillers per plant and number of spikes per m<sup>2</sup>. The nitrogen doses × sulfur management interaction was significant for number of

tillers per plant. Interaction between cultivars  $\times$  nitrogen doses were observed for number of spicules per spikes and number of seeds per spike (Table 1).

Table 1. Analysis of variance (mean square) of plant height (PH); number of tillers per plant (NTP); number of spicules per spike (NSS); height insert of main spike (HMS); number of seeds per spike (NSES); number of spikes per m<sup>2</sup> (NS); weight per 1000 seeds (WTS); hectoliter weight (HW); and seeds yield (SY) for wheat cultivars ('TBIO Sintonia', 'TBIO Sinuelo' e 'Jadeite 11'), as affected by nitrogen doses (0; 22.5; 45; 67.5 e 90 kg ha<sup>-1</sup>) and sulfur management (with and without). Capão do Leão, RS, UFPel, 2017

Source	D.F.	Mean Square <sup>(1)</sup>								
		PH	NTP	NSS	HMS	NSES	NS	WTS	HW	SY
Cultivar (C)	2	29.74 <sup>ns</sup>	0.25 <sup>ns</sup>	24.70*	38.82 <sup>ns</sup>	155.36*	15667.67 <sup>ns</sup>	92.36*	184.35*	2585021.00*
Sulfur (S)	1	35.75 <sup>ns</sup>	0.08 <sup>ns</sup>	0.16 <sup>ns</sup>	27.61 <sup>ns</sup>	16.71 <sup>ns</sup>	1034.88 <sup>ns</sup>	2.56*	0.08 <sup>ns</sup>	7852.47 <sup>ns</sup>
Doses (D)	4	57.45*	0.46*	4.26*	39.73*	38.74 <sup>ns</sup>	52203.68*	0.95 <sup>ns</sup>	1.16 <sup>ns</sup>	4665431.00*
Blocks	3	186.62	0.73	2.63	154.75	76.61	46377.68	2.93	9.28	1159173.00
C $\times$ S	2	47.91 <sup>ns</sup>	0.07 <sup>ns</sup>	0.24 <sup>ns</sup>	45.11*	17.69 <sup>ns</sup>	18505.54 <sup>ns</sup>	0.51 <sup>ns</sup>	4.00 <sup>ns</sup>	141619.00 <sup>ns</sup>
D $\times$ S	4	35.92 <sup>ns</sup>	0.24*	0.44 <sup>ns</sup>	31.08 <sup>ns</sup>	44.22 <sup>ns</sup>	21831.03 <sup>ns</sup>	0.82 <sup>ns</sup>	1.35 <sup>ns</sup>	206129.10 <sup>ns</sup>
C $\times$ D	8	24.90 <sup>ns</sup>	0.22*	1.57 <sup>ns</sup>	18.56 <sup>ns</sup>	50.59*	22717.77*	0.89 <sup>ns</sup>	1.86 <sup>ns</sup>	226610.50 <sup>ns</sup>
C $\times$ D $\times$ S	8	12.98 <sup>ns</sup>	0.12 <sup>ns</sup>	2.22*	9.53 <sup>ns</sup>	49.08*	13513.00 <sup>ns</sup>	0.65 <sup>ns</sup>	1.17 <sup>ns</sup>	163311.40 <sup>ns</sup>
Error	87	15.67	0.09	0.81	13.69	19.65	10716.92	0.45	1.49	168680.50
Average	-	78.26	0.84	13.07	71.28	26.60	569.85	33.03	79.39	3711.67
CV (%)	-	5.05	36.25	6.91	5.19	16.66	18.16	2.04	1.53	11.06

Note. <sup>(1)</sup> Mean square: \* and <sup>ns</sup>: significant at the 5% probability level and not significant, respectively; CV: coefficient of variation

Significant interaction between the factors revealed that treatments combinations provoke different effects on characters, especially because multiple genes control quantitative characters, and the environment strongly influence on these genes expression (Figure 1). In this way, nitrogen doses on top dressing, sulfur management, and the cultivar, are all factors that can cause differences on characters expression, and is necessary to slice into simple effects to obtain reliable results about the characters observed.

Nitrogen deficiency affects plant biomass production and efficiency on using solar radiation, influencing on yield components results (Heinemann et al., 2006; Xu et al., 2012; Ferrari et al., 2016). In contrast, nitrogen supplying to increase yield may also change plant architecture and improve photo-assimilates synthesis, such as starch and lipids, since the greater photosynthesis rates, the higher the respiration and cells multiplication and differentiation (Neumann, 2005). The processes of synthesis, partition and accumulation of photo-assimilates in plants are genetically controlled and influenced by environmental factors, like CO<sub>2</sub>, temperature, leaf area index, plant nutrition, water supply, between others (Donald & Hamblin, 1976).

Well-supplied plants tend to produce seeds with bigger size and weight, better embryo and cotyledons formation, and dry matter accumulation (Teixeira Filho et al., 2010; Marcos Filho, 2015; Szareski et al., 2018). The quadratic model is the one adjusted to height plant results, with high coefficient of determination ( $R^2 = 0.93$ ) (Figure 2a). Greater results were observed for the nitrogen dose of 58.8 kg ha<sup>-1</sup>, decreasing after that. Theoretical height increment of 4.6% was observed for plants submitted to this treatment, when compared to plants that did not received any nitrogen source.

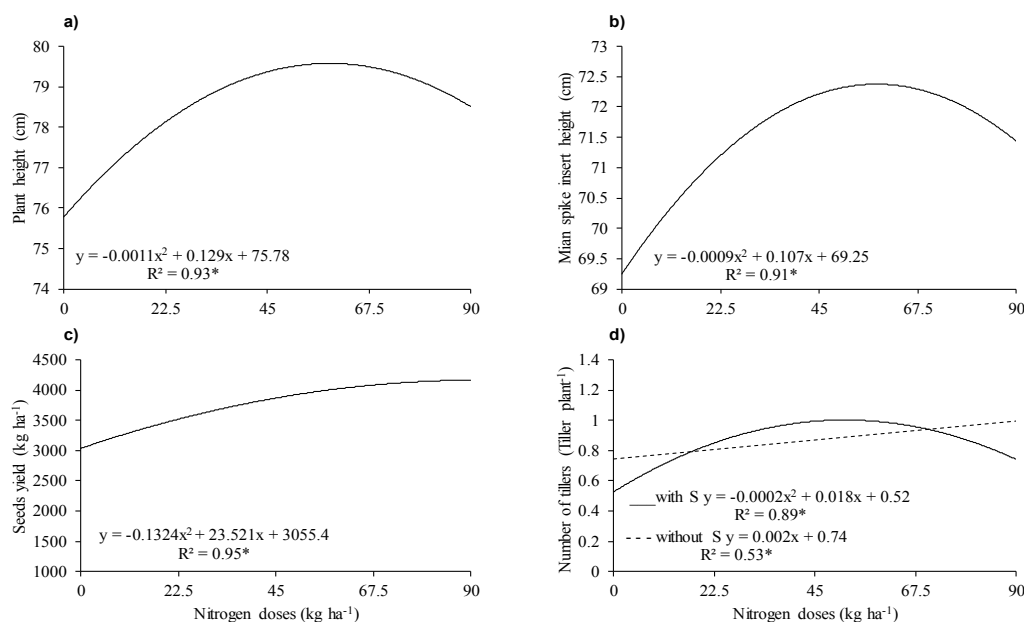


Figure 2. Plant height (a), Height insert of main spike (b) and seeds yield (c) for five nitrogen doses (0.0, 22.5, 45.0, 67.5 and 90.0 kg ha<sup>-1</sup>); and number of tillers per plant (d) for interaction between nitrogen doses × sulfur management. (\*5% significant level and <sup>ns</sup> not significant)

Plant height is a character influenced by environment (Souza et al., 2003), plants density (Brachtvogel et al., 2012), and, mainly, by availability of nitrogen in soil (Repke et al., 2013). The higher plant height values observed in rich nitrogen soils could be related to the higher mineral supply, which stimulate stems elongation.

However, excessively tall plants are not desirable, because they also are more predisposed to lodging. Similar results were recorded by Zagonel and Fernandes (2007), which observed a linear increase in plants height due to higher nitrogen doses; in the same way, Espindula et al. (2010) observed that increasing nitrogen doses linearly stimulates stems elongation.

The character number of tiller was influenced by the interaction between nitrogen doses and sulfur management. When sulfur was spread, the number of tiller per plant was adjusted to the quadratic trend curve (Figure 2d). Maximum efficiency was found on the dose of 51.7 kg ha<sup>-1</sup>, achieving about one tiller per plant, and decreasing after that. The number of tillers per plant increment is 48% higher than treatments without nitrogen (0.5 tillers plant<sup>-1</sup>).

Treatments without sulfur fertilization had the number of tiller per plant increased with higher nitrogen doses (Figure 2d). The increment, comparing to plants without nitrogen fertilization, is of 7.8, 14.6, 20.4 and 25.5% for the doses of 22.5, 45.0, 67.5 and 90.0 kg ha<sup>-1</sup>, respectively; with an average number for the higher dose of one tiller per plant.

In relation to the interaction between nitrogen doses × sulfur management, there was no significant difference for the nitrogen doses of 0.0, 22.5, 45.0 and 67.5 kg ha<sup>-1</sup>; however, the dose of 9.0 kg ha<sup>-1</sup> of nitrogen with no sulfur fertilization resulted in higher number of tiller per plant (Table 2).

This study revealed the influence of nitrogen and sulfur fertilization on wheat tillering, as well as the effect of both fertilizers combined, similarly to reported by Rodrigues (2002). Plants grown in soil with sulfur fertilization achieved maximum tillering (one tiller per plant) combined with 51.751,7 kg ha<sup>-1</sup> of nitrogen; and with no sulfur fertilization, the dose of 90 kg ha<sup>-1</sup> resulted in greater results (one tiller per plant). Thus, high doses of one of the fertilizers may induce lower availability to plants of the other fertilizer.

The cultivars presented interaction with nitrogen doses (Figure 3a). The number of tillers per plant for ‘TBIO Sintonia’ and ‘Jadeite 11’ has adjusted to the quadratic trend curve, achieving maximum tillering with 48.6 and 42.8 kg ha<sup>-1</sup> of nitrogen, and 40 and 12% of increment on number of tiller, respectively, comparing to plants without nitrogen fertilization (Figure 3a).

Table 2. Number of tillers per plant (tiller plant<sup>-1</sup>) in function of nitrogen doses (0.0, 22.5, 45.0, 67.5 and 90.0 kg ha<sup>-1</sup>) and sulfur management (with and without)

Doses (kg ha <sup>-1</sup> )	Number of tillers per plant	
	Sulfur management	
	Sulfur	No Sulfur
0.0	0.57 A*	0.66 A
22.5	0.75 A	0.91 A
45.0	1.03 A	0.93 A
67.5	1.01 A	0.82 A
90.0	0.71 B	1.02 A
CV (%)	36.25	

Note. \* Means followed by the same capital letter in the column do not differ by Tukey test ( $p < 0.05$ ).

For the cultivar 'TBIO Sinuelo', the number of tillers per plant increased linearly to high nitrogen doses. Increment of 55% was observed in plants grown in soil with 90 kg ha<sup>-1</sup> of nitrogen, comparing to plants without nitrogen fertilization (Figure 3a).

The cultivar 'Jadeíte 11' presented higher tillering for nitrogen doses of 0.00 and 22.5 kg ha<sup>-1</sup>, not differing from 'TBIO Sintonía'. Cultivars did not differ for nitrogen doses of 45.0 and 67.5 kg ha<sup>-1</sup>. The cultivar 'TBIO Sinuelo' revealed greater number of tillers per plant for 90.0 kg ha<sup>-1</sup> of nitrogen, not differing from 'Jadeíte 11' (Table 3).

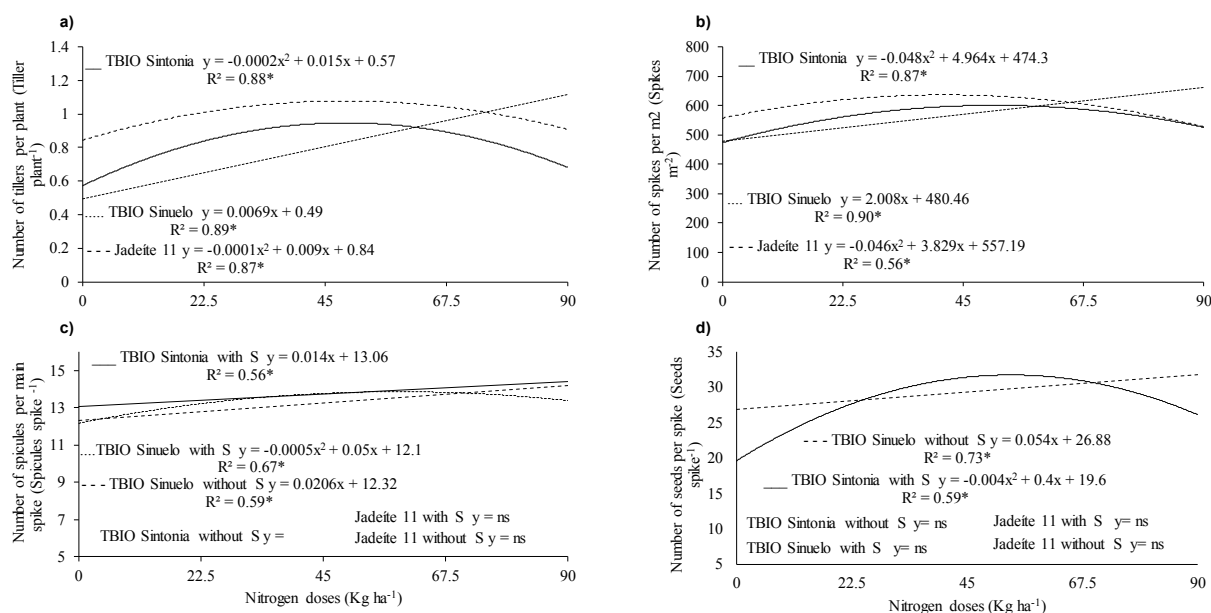


Figure 3. Interaction between nitrogen doses  $\times$  cultivar for the number of tiller per plant (a) and number of spikes per m<sup>2</sup> (b); and interaction between nitrogen doses  $\times$  sulfur management for the number of spicules per spike (c) and number of seeds per spike (d). (\*5% significant level and <sup>ns</sup> not significant)

The number of tillers per plant is an important character indirectly related to yield, especially if most of tillers are fertile. Nitrogen availability during the beginning of growing season is essential, so that the primary tillers are not omitted. Due to this fact, nitrogen restriction during growing season reduces the number of tillers per m<sup>2</sup>, reflecting on seeds yield decreases.

Table 3. Number of tillers per plant (tillers plant<sup>-1</sup>) and number of spikes per m<sup>2</sup> (spikes m<sup>-2</sup>) as function of nitrogen doses and wheat cultivars

Dose (kg ha <sup>-1</sup> )	Number of tillers per plant		
	Cultivars		
	'TBIO Sintonia'	'TBIO Sinuelo'	'Jadeíte 11'
0.0	0.61 AB*	0.42 B	0.82 A
22.5	0.76 AB	0.67 B	1.06 A
45.0	0.96 A	0.9 A	1.07 A
67.5	0.95 A	1.02 A	0.77 A
90.0	0.65 B	1.02 A	0.93 AB
CV (%)	36.25		
Dose (kg ha <sup>-1</sup> )	Number of spikes per m <sup>2</sup>		
	Cultivars		
	'TBIO Sintonia'	'TBIO Sinuelo'	'Jadeíte 11'
0.0	483.43 A*	458.50 A	532.53 A
22.5	546.87 AB	533.75 B	668.66 A
45.0	587.56 A	591.93 A	637.72 A
67.5	616.87 A	637.43 A	553.98 A
90.0	513.18 A	632.62 A	552.75 A
CV (%)	18.16		

Note. \* Means followed by the same capital letter in the column do not differ by Tukey test ( $p < 0.05$ ).

In addition, the effects of using nitrogen fertilization are different on wheat cultivars from different genetic basis, and some traits may present different effects on growing season, plant architecture, and yield potential; these effects may interfere on nitrogen absorption, assimilation, and conversion for seeds production (Sangoi et al., 2007).

The character number of tillers per plant is influenced by many factors, including genetics, which can explain the high coefficient of variation value (Pimentel Gomes, 2009). In a study developed by Abati et al. (2017), in which they have evaluated number of tillers per plant, a similar coefficient of variation was reported (29.3%).

In weather conditions of South of Brazil, tillering is a desirable character for winter cereals, and demands high phenotypic plasticity to respond to environmental changes. This plasticity allows plants to adapt to different densities at the field (Almeida et al., 2004; Ferrari et al., 2016).

According to Valério et al. (2008), nitrogen fertilization is important for tillers to emerging, developing, and surviving; consequently, it also influence on plants yield. Though wheat plants are able to balance out plants population changes through tillers emergence, the percentages of fertile and total tillers are variable among genotypes (Valério et al., 2008). Otherwise, the number of tillers per plant and their development is correlated with the intra-specific competition for environmental factors (Caires et al., 2001).

Sulfur is a crucial nutrient demanded by plants to ensure tillers growing. The combination between sulfur and amino acids makes the called "building blocks", from which proteins are synthetized. Sulfur translocation rates in plant are low, in a way that young parts of plant are the first affected by sulfur deficiency. To overcome this problem, continuously supplying through multiple sulfur spreads must to be performed to provide a good plant growing during demand peaks.

The number of spicules per main spike for the cultivar 'TBIO Sintonia' with sulfur has adjusted to a linear and crescent tendency, improving with nitrogen doses (Figure 3c). With the dose of 90 kg ha<sup>-1</sup>, the number of spicules per spike was 14.3; this value is 8.7% higher than treatments without nitrogen fertilization. For the cultivar 'TBIO Sinuelo', with sulfur fertilization, the number of spicules per spike has adjusted to a quadratic tendency curve (Figure 3c). The maximum efficiency was achieved at the dose of 57.3 kg ha<sup>-1</sup>, with 13.8 spicules in average, with 11.8% more spicules until dose of maximum efficiency (57.3 kg ha<sup>-1</sup>).

For the cultivar 'TBIO Sinuelo', in traits without sulfur fertilization, a linear and crescent tendency was observed among nitrogen doses (Figure 3c). For the amount of 90 kg ha<sup>-1</sup> of nitrogen, a number of 14.1 spicules per spike was obtained, which one is 12.6% superior than treatments without nitrogen fertilization. For 'TBIO Sintonia' without sulfur fertilization, and for 'Jadeíte 11' with and without sulfur fertilization, the number of spicules per spike did not presented differences among nitrogen doses.

The interaction between wheat cultivars  $\times$  nitrogen doses  $\times$  sulfur management is represented at the Table 4. For the character number of spicules per main spike, in plants with sulfur fertilization and nitrogen at the doses of 0.0, 45.0, and 67.5 kg ha<sup>-1</sup>, the cultivars did not differ (Table 4). At the nitrogen doses of 22.5 and 67.5 kg ha<sup>-1</sup>, 'TBIO Sinuelo' and 'TBIO Sintonia' obtained a greater number of spicules per spike than 'Jadeite 11'. Plants without sulfur fertilization, at the nitrogen doses of 22.5 and 90.0 kg ha<sup>-1</sup>, the cultivars did not differ from each other for the number of spicules per main spike. For the nitrogen doses of 0.0, 45.0, and 67.5 kg ha<sup>-1</sup>, the higher number of spicules per spike was observed for the cultivars 'TBIO Sintonia' and 'TBIO Sinuelo'.

Comparing sulfur management and nitrogen doses for each cultivar, sulfur fertilization did not influence on number of spicules per spike for 'TBIO Sintonia'; while for the cultivar 'TBIO Sinuelo', at the dose of 22.5 kg ha<sup>-1</sup> of nitrogen, the number of spicules per spike improved with sulfur fertilization. The cultivar 'Jadeite11' produced higher number of spicules per spike with sulfur fertilization and nitrogen at the dose of 67.5 kg ha<sup>-1</sup> (Table 4).

An appropriate nitrogen supplying for plants during grain filling is fundamental to improve yield; and some yield components, such as number of spikes per area and number of spicules per spike, are strongly influence by the moment that nitrogen fertilization is made (Braz et al., 2006). A study developed by Benett et al. (2011) reported that the number of spicules per spike was highly influenced by the stage that the nitrogen fertilization was performed. In this way, the most crucial and nitrogen demanding stage is between initial developing and floral primordial differentiation. The nitrogen doses improve the number of spicules per spike according to a quadratic tendency (Filho et al., 2007).

According to Bredemeier and Mundstock (2001), nitrogen fertilization should be performed between the emergence and the emission of seventh leaf from main stem. In the beginning of this period, nitrogen is demanded for a good establishment of differentiated spicules number and, consequently, number of seeds per spike.

The height insert of main spike has adjusted to a quadratic tendency curve (Figure 2b). The insert height was increased until the maximum point, which was at the nitrogen dose of 58.0 kg ha<sup>-1</sup>, with 72.3cm, decreasing after that point. No nitrogen fertilization resulted in 4.2% of insert height reduction, comparing to the maximum efficiency dose. Even with a decreasing tendency, the nitrogen dose of 90.0 kg ha<sup>-1</sup> showed 3.1% of increasing on height insert of main spike than plants without nitrogen fertilization.

Table 4. Number of spicules per spike and number of seeds per spike for nitrogen doses (kg ha<sup>-1</sup>), cultivars and sulfur management

Number of spicules per spike						
Doses (kg ha <sup>-1</sup> )	'TBIO Sintonia'		'TBIO Sinuelo'		'Jadeite 11'	
	With sulfur	Without sulfur	With sulfur	Without sulfur	With sulfur	Without sulfur
0	13.06 A $\alpha$ *	13.66 A $\alpha$	11.87 A $\alpha$	12.26 AB $\alpha$	12.15 A $\alpha$	11.44 B $\alpha$
22.5	12.93 AB $\alpha$	13.30 A $\alpha$	13.95 A $\alpha$	12.23 A $\beta$	12.11 B $\alpha$	13.10 A $\alpha$
45.0	14.21 A $\alpha$	13.72 AB $\alpha$	13.41 A $\alpha$	13.97 A $\alpha$	12.9 A $\alpha$	12.23 B $\alpha$
67.5	14.48 A $\alpha$	14.22 A $\alpha$	13.57 AB $\alpha$	14.25 A $\alpha$	12.80 B $\alpha$	11.12 B $\beta$
90.0	13.94 A $\alpha$	13.97 A $\alpha$	13.57 AB $\alpha$	13.57 A $\alpha$	11.72 B $\alpha$	12.54 A $\alpha$
CV(%)	6.91					
Number of seeds per spike						
Doses (kg ha <sup>-1</sup> )	TBIO Sintonia		TBIO Sinuelo		Jadeite 11	
	With sulfur	Without sulfur	With sulfur	Without sulfur	With sulfur	Without sulfur
0	20.95 A $\beta$ *	31.71 A $\alpha$	23.99 A $\alpha$	27.89 A $\alpha$	23.41 A $\alpha$	25.19 A $\alpha$
22.5	23.19 A $\alpha$	22.76 A $\alpha$	29.68 A $\alpha$	26.40 A $\alpha$	23.42 A $\alpha$	27.46 A $\alpha$
45.0	37.01 A $\alpha$	28.02 A $\beta$	25.57 B $\alpha$	30.22 A $\alpha$	23.85 B $\alpha$	26.92 A $\alpha$
67.5	27.99 A $\alpha$	25.56 AB $\alpha$	27.78 A $\alpha$	29.89 A $\alpha$	26.02 A $\alpha$	22.06 B $\alpha$
90.0	26.67 AB $\alpha$	23.90 B $\alpha$	31.09 A $\alpha$	32.29 A $\alpha$	22.82 B $\alpha$	24.34 B $\alpha$
CV(%)	16.66					

Note. \*Means followed by the same capital letters in the lines for cultivars within sulfur management; and Greek letters in the lines for sulfur management within cultivar do not differ by the Tukey test ( $p < 0.05$ ).



For the character height insert of main spike, cultivars did not present differences without sulfur fertilization (Table 5). However, for the treatments with sulfur fertilization, the cultivar 'TBIO Sintonia' presented greater height insert of main spike than 'Jadeíte 11', and did not differ from 'TBIO Sinuelo'. Comparing cultivars within sulfur management, 'TBIO Sintonia' achieved higher height insert of main spike without sulfur fertilization; while for 'TBIO Sinuelo' and 'Jadeíte 11', no differences were observed with or without sulfur fertilization (Table 5).

The height insert of main spike is an important character, since reduces chances of plants lodging. Plant height is influenced by sowing density, which one affects the height insert of main spike (Gross et al., 2012).

Table 5. Height insert of main spike (cm) for cultivars and sulfur management

Cultivar	Height insert of main spike (cm)	
	Sulfur management	
	With sulfur	Without sulfur
'TBIO Sintonia'	71.00 aB*	73.65 aA
'TBIO Sinuelo'	69.54 aA	71.19 abA
'Jadeíte 11'	71.87 aA	70.45 bA
CV (%)	5.19	

Note. \*Means followed by the same lower case letter in the column and capital letter in the line do not differ by Tukey test ( $p < 0.05$ ).

The increment of height insert of main spike until the maximum efficiency dose ( $58.0 \text{ kg ha}^{-1}$ ) could be related to the fact that nitrogen is an internodes elongation booster for wheat plants, and this dose is great to improve morphologic development, allowing bigger photosynthetically active area and photoassimilates conversion.

The character number of seeds per spike for the cultivar 'TBIO Sintonia' with sulfur fertilization has adjusted to a quadratic tendency (Figure 3d). Number of seeds per spike increased until the maximum efficiency point, which was with nitrogen dose of  $53.5 \text{ kg ha}^{-1}$ , presenting 31.8 seeds per spike. Comparing to traits without nitrogen fertilization, 38% of seeds per spike improvement was observed. For the cultivar 'TBIO Sinuelo', without sulfur fertilization, a linear and crescent tendency was observed for the nitrogen doses (Figure 3d). This increment represents 15.2% more seeds per spike between the higher nitrogen dose ( $90.0 \text{ kg ha}^{-1}$ ) and the lower dose ( $0.0 \text{ kg ha}^{-1}$ ). For 'TBIO Sintonia' without sulfur fertilization, for 'TBIO Sinuelo' with sulfur fertilization, and for 'Jadeíte 11' with and without sulfur fertilization, the number of seeds per spike did not differ between nitrogen doses.

With the nitrogen dose of  $45.0 \text{ kg ha}^{-1}$ , a greater number of seeds per spike was obtained for the cultivar 'TBIO Sintonia' than for 'TBIO Sinuelo' and 'Jadeíte 11'. For the dose of  $90.0 \text{ kg ha}^{-1}$ , the cultivars 'TBIO Sintonia' and 'TBIO Sinuelo' obtained higher number of seeds per spike than 'Jadeíte 11' (Table 4).

Analyzing the treatments with and without sulfur fertilization within each cultivar for each nitrogen dose, for the cultivar 'TBIO Sintonia', at the nitrogen dose of  $45 \text{ kg ha}^{-1}$ , the number of seeds per spike was higher with sulfur fertilization. For the cultivars 'TBIO Sinuelo' and 'Jadeíte 11', there was no difference on the number of seeds per spike with any nitrogen dose, due to sulfur fertilization (Table 4).

High doses of nitrogen fertilization result in vigorous plant development, fundamental for meristems definition (Espindula et al., 2010); producing better values of yield components, especially number of seeds per spike (Benin et al., 2012; Szareski et al., 2018).

Different values were observed for number of seeds per spike, due to characteristic of this character, and to its interaction with environment. Braz et al. (2006) observed similar values to this study, ranging from 34 to 36.8 seeds. In addition, Cazetta et al. (2007) verified linear increment of triticale and wheat seeds with higher nitrogen fertilization doses.

The number of spikes per  $\text{m}^2$  was influenced by nitrogen doses. For the cultivar 'TBIO Sintonia', the number of spikes per  $\text{m}^2$  has adjusted to a quadratic tendency, achieving maximum response at the nitrogen dose of  $51 \text{ kg ha}^{-1}$ , with 600 spikes per  $\text{m}^2$  (Figure 3b). The increment observed in relation to treatments without nitrogen fertilization was of 21% more spikes per  $\text{m}^2$ .

For the cultivar ‘TBIO Sinuelo’, the number of spikes per m<sup>2</sup> has adjusted to a crescent linear tendency with increasing nitrogen doses (Figure 3b). The number of spikes per m<sup>2</sup> increased 8.6, 15.8, 22.0, and 27.3% in plants submitted to nitrogen fertilization at the doses of 22.5, 45.0, 67.5, and 90.0 kg ha<sup>-1</sup>, respectively, in comparison to the ones without nitrogen fertilization, achieving about 660 spikes m<sup>2</sup> with 90.0 kg ha<sup>-1</sup>. The cultivar ‘Jadeíte 11’ has adjusted to a quadratic tendency (Figure 3b), achieving maximum efficiency for spikes production with 41.4 kg ha<sup>-1</sup> of nitrogen fertilization, resulting in 636 spikes per m<sup>2</sup>. This increment represents 12.4% more spikes per m<sup>2</sup> than plants without nitrogen fertilization.

The interaction slicing of the character number of spikes per m<sup>2</sup> revealed no differences between cultivars for the nitrogen doses of 0.0, 45.0, 67.5, and 90 kg ha<sup>-1</sup>. However, for the dose of 22.5 kg ha<sup>-1</sup>, ‘Jadeíte 11’ presented the highest number of spikes per m<sup>2</sup>, not differing from ‘TBIO Sintonia’ (Table 3).

For the character weight per 1000 seeds, the cultivar ‘TBIO Sinuelo’ has resulted in higher value, followed by ‘Jadeíte 11’ and by ‘TBIO Sintonia’ (Table 6).

Table 6. Weight per 1000 seeds (g), hectoliter weight (kg hL<sup>-1</sup>), and seeds yields (kg ha<sup>-1</sup>) for wheat cultivars

Cultivars	WTS (g)	HW (kg hL <sup>-1</sup> )	Yield (kg ha <sup>-1</sup> )
‘TBIO Sintonia’	31.73 c*	76.90 b	3448.00 c
‘TBIO Sinuelo’	34.70 a	80.34 a	3956.00 a
‘Jadeíte 11’	32.66 b	80.90 a	3730.00 b
CV (%)	2.04	1.53	11.06

Note. \* Means followed by the same capital letter in the column do not differ by Tukey test ( $p < 0.05$ ).

Differences for the character weight per 1000 seeds among cultivars may be due to genetic effects (Freitas et al., 2007), since each cultivar has particular ways to use and take advantage of the nutrients for seeds filling. Soil and climatic conditions are other important factors, mainly temperature and humidity, especially during seeds maturation stage (Costa et al., 2013).

For treatments with sulfur management, the character weight per 1000 seeds was higher without sulfur fertilization (Table 7). Sulfur is an amino acids component (cysteine and methionine). Therefore, its deficiency results in lower amount of these amino acids, as well as the proteins composed by them. Because of this, not enough sulfur supplying results in difficulty for plants to assimilate nitrogen in proteins, been the nitrogen accumulated as amines, amides, and soluble amino acids. Thus, a good balance between nitrogen and sulfur rates is fundamental to plant nutrition and growing, which means that higher rates of one of these nutrients may lead to lower availability of the other nutrient to plant, impairing plant yield.

The results of weight seeds due to nitrogen fertilization are contradictory, since some studies reported positive effects, like for crescent nitrogen doses in maize (Sangoi et al., 2011); while other studies reported decreasing of weight per 1000 seeds with nitrogen doses increasing (Favarato et al., 2012).

Table 7. Weight per 1000 seeds (g) due to sulfur management

Sulfur management	Weight per 1000 seeds (g)
With sulfur	32.89 b*
Without sulfur	33.18 a
CV (%)	2.04

Note. \* Means followed by the same capital letter in the column do not differ by Tukey test ( $p < 0.05$ ).

According to Teixeira Filho et al. (2010), the non-influence of nitrogen fertilization on weight per 1000 seeds could be due to the highest number of seeds per spike, which would cause more competition for fotoassimilates, reducing unitary seeds weight.

Cultivars presented differences for the character hectoliter weight. The cultivars ‘Jadeíte 11’ and ‘TBIO Sinuelo’ performed higher results, comparing to ‘TBIO Sintonia’ (Table 6). According to Alvarez (2004), hectoliter weight is not influenced by sulfated fertilization.

These study results suggest greater seeds filling for the cultivars 'Jadeite 11' and 'TBIO Sinuelo'. However, higher number of seeds per spike was observed for the cultivar 'TBIO Sinuelo'. In this way, yield was influenced by the yield components weight per 1000 seeds, hectoliter weight, and number of seeds per spike.

Regarding to plants yield, greater values were observed for the cultivar 'TBIO Sinuelo', which one has 12% more yield than 'TBIO Sintonia', which presented lowest average yield (Table 6).

The character seeds yield of wheat plants submitted to different nitrogen doses has adjusted to a quadratic model, with high coefficient of determination ( $R^2 = 0.95$ ) (Figure 2c). Yield values achieved maximum efficiency for the dose of 88.8 kg ha<sup>-1</sup>, with 4099 kg ha<sup>-1</sup>.

Studies performed by Zhao et al. (1999), and Tea et al. (2007), showed inconclusive results for interaction between sulfur and nitrogen fertilization. The elementary sulfur need to be oxidized to become available for plants (Horowitz, 2006), either by biotic or abiotic factors. Osório Filho et al. (2007), and Rheinheimer et al. (2005) did not observe response of wheat plants to crescent sulfur doses at sowing, attributing the results to fast sulfur lixiviation to deeper layers at soil, been out of roots reach, since the study was conducted in sandy soil (Nogueira & Melo, 2003; Osório Filho et al., 2007).

Nitrogen fertilization has showed influence on plants yield, according results presented in this study. The increment on seeds yield of wheat plants submitted to nitrogen fertilization in the dose of 88.8 kg ha<sup>-1</sup>, which was the dose of maximum efficiency, was of 25.4% more than treatments without nitrogen fertilization. The quadratic response for yield agree with results reported for wheat crop (Barbosa et al., 2016; Coelho et al., 1998; Espindula et al., 2009; Teixeira Filho et al., 2007). Heinemann et al. (2006) has found response until the nitrogen dose of 156 kg ha<sup>-1</sup>, with 6,472 kg ha<sup>-1</sup> of yield for irrigated wheat plants. Usually, best yields are observed with nitrogen fertilization doses among 70 to 120 kg ha<sup>-1</sup> (Espindula et al., 2010; Teixeira Filho et al., 2008).

Although yield responses of wheat plants to nitrogen fertilization differ for different cultivars, climatic and soil conditions, among other factors, most of the results showed that nitrogen fertilization is always benefic for yield than no nitrogen fertilization (Vieira et al., 1995). Overall, sulfur has low influence on yield components and yield, because this nutrient has low mobility on plant, and the soil where the study was carried out presented adequate sulfur amount before sowing. The cultivars, according to its genetic bases, may respond differently to fertilization management. In this study, the cultivar 'TBIO Sinuelo' performed better results of yield components and yield with the increase of nitrogen fertilization doses than the cultivars 'TBIO Sintonia' and 'Jadeite 11'.

#### 4. Conclusions

Nitrogen fertilization has different effects on wheat cultivars. The cultivar 'TBIO Sinuelo' presents greater seeds yield with improvement of nitrogen fertilization doses. High nitrogen doses provide increasing of wheat seeds yield. Nitrogen doses influence differently on yield components of wheat. Number of tillers per plant is correlated with seeds yield. Physical attributes of wheat seeds are not influenced by nitrogen doses increasing. Sulfur fertilization promotes tillering and increment of number of spicules per spike. The cultivar 'TBIO Sinuelo' presents higher weight per 1000 seeds, followed by 'Jadeite 11', and 'TBIO Sintonia'.

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