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ARTICLE

Is the physiological potential of oilseed rape influenced by fertilization with nitrogen and sulfur?

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ABSTRACT: The productivity of the oilseed rape crop is responsive to the application of nitrogen (N) and the effect of N is sulfur (S)-dependent. In addition, storage may influence seed vigor. The aim of the present study was to assess the effect of fertilization with nitrogen and sulfur on the physiological quality of rapeseeds. All combinations of four doses of N (0, 40, 80, and 120 kg.ha^{-1}) and five of S (0, 30, 60, 90, and 120 kg.ha^{-1}) were evaluated with four replications each distributed in a randomized block design in a distroferric Red Latosol soil. The effects of the nutrients and their combinations on the physiological potential of rapeseeds were evaluated even immediately after harvest and 180 days of storage through the germination test, accelerated aging, field emergence and seedling performance. The application of N associated with S had a positive effect on the physiological quality of the seeds. The application of the 120 kg.ha^{-1} dose of N and the 80 kg.ha^{-1} dose of S promoted the highest germination and vigor of the seeds. Seed storage for a period of 180 days led to a drastic reduction in the physiological potential of the rapeseeds.

Index terms: *Brassica napus*, crop technology, seed vigor, storage.

O potencial fisiológico das sementes de canola é influenciado pela adubação com nitrogênio e enxofre?

RESUMO: A produtividade da canola é responsiva à aplicação de N e o efeito de N depende de S. Além disso, o armazenamento pode influenciar o vigor das sementes. O objetivo do presente estudo foi avaliar o efeito da fertilização com nitrogênio e enxofre na qualidade fisiológica de sementes de canola. Todas as combinações de quatro doses de N (0, 40, 80 e 120 kg.ha^{-1}) e cinco doses de S (0, 30, 60, 90 e 120 kg.ha^{-1}) foram avaliadas com quatro repetições em delineamento de blocos casualizados, em um solo Latossolo Vermelho distroférrico. Os efeitos dos nutrientes e suas combinações sobre o potencial fisiológico das sementes de canola foram avaliados imediatamente após a colheita e após 180 dias de armazenamento pelo teste de germinação, envelhecimento acelerado, emergência em campo e desempenho de plântulas. A aplicação de N associada com S apresentou um efeito positivo na qualidade fisiológica de canola. A aplicação da dose de 120 kg.ha^{-1} de N, associada à dose de 80 kg.ha^{-1} de S, promoveu maior germinação e vigor das sementes de canola. O armazenamento das sementes por um período de 180 dias proporcionou uma redução acentuada no potencial fisiológico das sementes de canola.

Termos para indexação: *Brassica napus*, tecnologia de produção, vigor de sementes, armazenamento.

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INTRODUCTION

Oilseed rape is a winter crop developed from the genetic improvement of rapeseed (*Brassica napus* L.) (Kaefer et al., 2014). It has great economic importance and stands out as the third most-produced oilseed in the world because of the high oil quality and content (34% to 38%) and high protein content (24% to 27%) of its grains (Chavarria et al., 2011). Commercial crop is geographically concentrated in temperate regions, mainly in latitudes higher than 35 °N or S. Air temperature is the most important environmental variable regulating oilseed rape growth and development, with the optimal temperature for oilseed rape growth being around 20 °C and its upper and lower limits being 12 and 30 °C, respectively (Dalmago et al., 2011). In addition to thermal requirements, attention should be focused on the quality of the seeds that are used to grow this crop and are, therefore, the main input to its modern agriculture.

Since the nutritional status of the mother plant has an important influence on the physiological component of seed quality and seed longevity during storage, the combination of external factors and nutritional conditioning from the mother plant should provide seeds with all the essential mineral elements they need to grow (Nonogaki, 2006). Although the roles of the most essential elements, such as N and S, in plant biochemical functions are known, there is a lack of knowledge about various aspects of how exogenous availability of such mineral elements affects germination and, consequently, seedling establishment. Whether and how nutrient absorption during seed development influences germination and establishment in species of plants that produce small seeds and have low reserves, such as oilseed rape, also remains an open question (Dubousset et al., 2010; Eggert and Von Wirén, 2016).

Despite its high mineral N uptake capacity, the rapeseed is characterized by a low N use efficiency (NUE) because only 50% of the N absorbed by the plant is present in the seeds at harvest (Avice and Etienne, 2014). This is due to the fact that N is easily lost through leaching, volatilization, and denitrification in the soil (Nascente et al., 2011). In addition to grain yield, N affects the production of aerial dry matter, leaf area, number and dry mass of siliquae per plant, and oil content of oilseed rape grains (Kaefer et al*.,* 2014).

Despite the fact that the success of stand establishment depends primarily on the quality of the seeds used in planting, and the consensus on the importance of mineral nutrition in the development of vigorous seeds, there is still a need for more data on the extended effects of nutrients on the post-seed-harvest, especially the storage stage. According to Sano et al. (2016), seed longevity depends on the initial seed moisture content, relative humidity, temperature, and oxygen pressure; the viability of seeds gradually decreases during storage because of deterioration in these conditions, which may lead to irreversible damage. High seed moisture content combined with high temperature accelerates the natural degradation processes, which lead to seeds rapidly vigor losses and, soon after, their germination capacity (Walters, 2015; Waterworth et al., 2015).

According to the importance of the oilseed rape crop and the importance of high quality of the seeds to achieve field performance, the aim of this research was answer the following question: could the fertilization with combined doses of N and S influence the physiological potential of rapeseeds, especially after storage?

MATERIAL AND METHODS

Oilseeds Crop Production: in this study, an experiment with the oilseed rape Hyola 433 was conducted during the winter of 2016 in the Experimental Farm of the School of Agricultural Sciences, *Universidade Federal da Grande Dourados* (UFGD), located in the municipality of Dourados, Mato Grosso do Sul state, Brazil (22 °14 ′ S, 54 °59 ′ W, altitude 434 m). The climate in the study area is of the 'Cwa' type of the Köppen classification system, and the soil is a distroferric Red Latosol. Chemical analysis of the soil was performed on a sample collected at a depth of 0−20 cm before corn harvest in the second crop of 2016, and the following results were obtained: M.O. = 28 g.dm^{-3}; pH (H_2O) = 5.72; P = 12.8 mg.dm^{-3}; K = 1.53 mmol$_c$.dm^{-3}; Al = 0; Ca = 5.34; Mg = 2.01 mmol$_c$.dm^{-3}; [+Al] = 6.5; TEB = 8.88; T = 15.3; and BS% = 57.7.

The sources of the elements were urea (45% N) and elemental S (99.5% S). Each plot was composed of seven 5-m-long rows spaced 0.40 m apart, taking up a total area of 14 m^2. At the sowing of the oilseed rape Hyola 433 seeds, 200 $kg.ha^{-1}$ of the 00-20-20 formula was laid down by hand in the furrows. Sowing occurred on April 21, 2016 at a depth of 2 to 3 cm. Seeds were harvested by hand from the plants that grew after sowing on August 11, 2016, from the three most central 5-m-long rows, with the total harvest area 6 m^2. Data on the local climatic conditions during the experimental period are shown in Figure 1.

To determine N and protein contents, 20 leaves per plot were collected at the initial budding stage. The fourth leaf from the apex to the base of the plant was selected for analysis. The leaves were washed with deionized water and dried in a forced-air oven at 65 °C. They were subsequently ground, and then their N content was determined through digestion with sulfuric acid by the Kejldahl method (Malavolta et al., 1997). Seed protein content was calculated as follow: seed protein = N x 6.25.

After harvest, seeds were sent to the Laboratory of Seed Technology of the UFGD. Once there, nonstandard seeds were discarded, and then homogeneous lots were established for each treatment. One half of the seeds from each treatment was placed in a paper bag and stored in a cold and dry chamber (15 °C, 45% RH) in the laboratory for 180 days; the other half was immediately submitted to the following assays and determinations.

Seed moisture content: it was assessed using the oven method at 105 ± 3 °C for 24 hours, using two samples from each lot, according to the Rules for Seed Testing (Brasil, 2009).

Seed germination: germination was assessed with four replications of 50 seeds per each field replicate in a Biochemical Oxygen Demand (BOD) incubator at 20 °C and under continuous white light. Germitest paper was used as the substrate and moistened with an amount of water equivalent to 2.5 times the weight of the dry substrate. Counts of germinating seeds were made seven days after sowing, according to the criteria established in Rules for Seed Testing (Brasil, 2009).

Field emergence: it was assessed in a greenhouse with 50% shading, where four replications of 50 seeds were sown in seedbeds filled with distroferric Red Latosol soil; the soil was irrigated as required. The number of seedlings that emerged was assessed 15 days after sowing, and the results were used to calculate the percentage of seeds that emerged (i.e. percent emergence) (Nakagawa, 1999).

Accelerated aging: it was performed with four replications of 50 seeds, which were placed on a stainless-steel tray inside a germination box with 40 mL of water at the bottom and kept in a BOD incubator at 41 °C for 24 hours. After this period, the seeds were subjected to the germination assay according to the description provided above. The percentage of normal seedlings that resulted from germination was assessed five days after sowing, according to the criteria of the Rules for Seed Testing (Marcos-Filho, 1999).

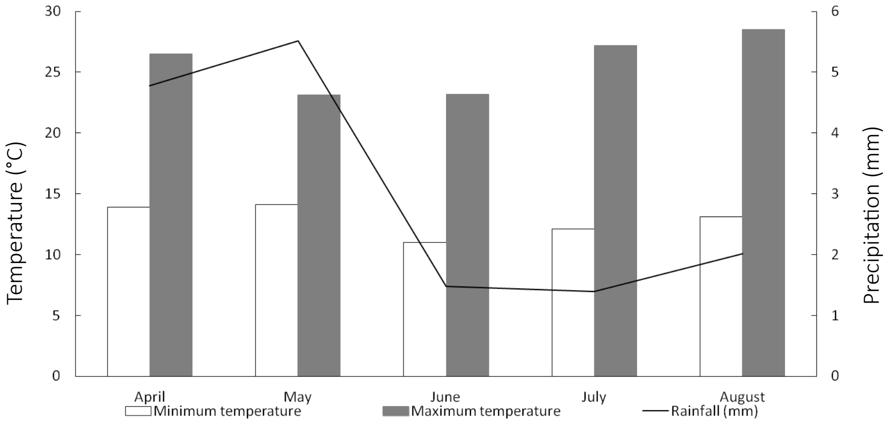


Figure 1. Monthly rainfall and mean temperatures during the growth period of the oilseed rape crop, 2016, in Dourados, Mato Grosso do Sul, Brazil. Source: Agrometereological Station of the Agrarian Sciences Experimental Farm.

Seedling length: it was assessed for four replications of 20 seeds that were placed in the upper third of the germitest paper and kept at 20 °C. Measurements (with a millimetric ruler) of the lengths of the aerial part of the plant (stem) and root were made seven days after sowing (Nakagawa, 1999).

Dry matter mass: it was determined using the normal seedlings from the seedling length assay. After the measurement of length, the seedlings were divided into different parts, placed in paper bags, and weighed to determine their fresh mass. They were subsequently kept in a forced-air oven at 40 °C for 48 hours. The samples were then removed from the oven and weighed to determine their dry mass; the results were expressed in grams (g).

Experimental Design and Statistical Analysis: the oilseed rape Hyola 433 was produced, in a randomized block experimental design with 20 treatments (with four replications each) in a 5 × 4 factorial arrangement, which consisted of four doses of N (0, 40, 80, and 120 $kg.ha^{-1}$) and five doses of S (0, 30, 60, 90, and 120 $kg.ha^{-1}$). The laboratory data were subjected to analyses of variance (ANOVA) for a completely randomized design, and regression analysis was performed between N and S concentrations and the dependent variable using SISVAR statistical software when the results of ANOVA were significant ($p < 0.05$). The surface response analysis was determined with SAEG, version 9.1 (SAEG, 2007).

RESULTS AND DISCUSSION

There was not adjustment of the response surface for the N × S interaction ($p < 0.05$) on the protein content of the rapeseeds and the average result was about 31.42%. This result is superior to those obtained in a study conducted in Toledo (Paraná state, Brazil) by Kaefer et al. (2014), who reported a mean protein content of 27% with the same dose of N (120 $kg.ha^{-1}$). The superior results of the present study may be related to the higher temperatures observed during seed maturation under the conditions of the experiment conducted in this study in Dourados (Mato Grosso do Sul state, Brazil), as the maximum temperature was 23.4 °C during the grain filling stage. Temperatures above 23 °C lead to a decrease in oil content and an increase in protein content in oilseed rape grains (Rathke et al*.,* 2006).

No difference was observed for productivity and one thousand-seed weight which presented an average of 980.8 $kg.ha^{-1}$ and 5.92 g, respectively. The mean moisture content in oilseed rape seeds produced with the different treatments in this study was 9.1% post-harvest and 9.7% after storage for 180 days under controlled environmental conditions of temperature and RH.

There was a significant effect of both the N × S interaction and the N dose × storage interaction on seed germination and there was a significant adjustment of the response surface for the interaction of factors ($p < 0.05$) (Figure 2). However, the application of the 80 $kg.ha^{-1}$ dose of N led to an increase in seed germination with increasing doses of S. The maximum percent germination (54%) was obtained with the 120 $kg.ha^{-1}$ dose of N and the 70 $kg.ha^{-1}$ dose of S (Figure 2A). Although there was a significant interaction between doses of N and seed storage, there was no linear regression fit for these data ($p > 0.05$) (Figure 2B). The analysis of the S × storage interaction showed that 71% of the recently harvested seeds germinated, whereas the maximum germination of the seeds stored for 180 days was 21% using the 59 $kg.ha^{-1}$ dose of S (Figure 2C). These results indicated that although RH and temperature were controlled, there was a reduction in the physiological potential of the oilseed rape seeds over 180 days of storage. Seed longevity decreases rapidly under storage conditions, one of the causes of which is an increase in cytoplasm fluidity, which promotes the irreversible formation of aggregations of denatured proteins (Sano et al*.*, 2016).

There was a significant effect of the interaction ($p < 0.05$) between the nutrients and storage on field emergence. For the seeds produced with N treatment, there was a significant regression fit between N dose and emergence only for seeds that were not stored, which indicated that seed vigor benefited from increasing doses of N at intervals of 0.1185 (Figure 3A). For the seeds produced with S treatment and subjected to storage, the minimum result was 2% emergence at the 114 $kg.ha^{-1}$ dose of S (Figure 3B). These results showed that storage was detrimental to seed vigor, but also indicated that fertilization with N and S had positive effects on the production of oilseed rape seeds with high physiological potential.

Field emergence allows the on-site assessment of seed performance to be carried out, and the results for this assay allowed us to infer that treatment with both nutrients was crucial for the production of vigorous oilseed rape seeds. During the grain filling stage, there is extensive synthesis and deposition of reserves; in oilseed rape specifically, reserves are comprised of 45−50% oil and 20−25% proteins, of which 60% are storage proteins. Thus, the unavailability of S may limit the content of S in seeds and, subsequently, the percentage of storage proteins they contain (Brunel-Muguet et al., 2015b), such as proteins rich in S like cruciferin (Cru4) (D'Hooghe et al., 2014).

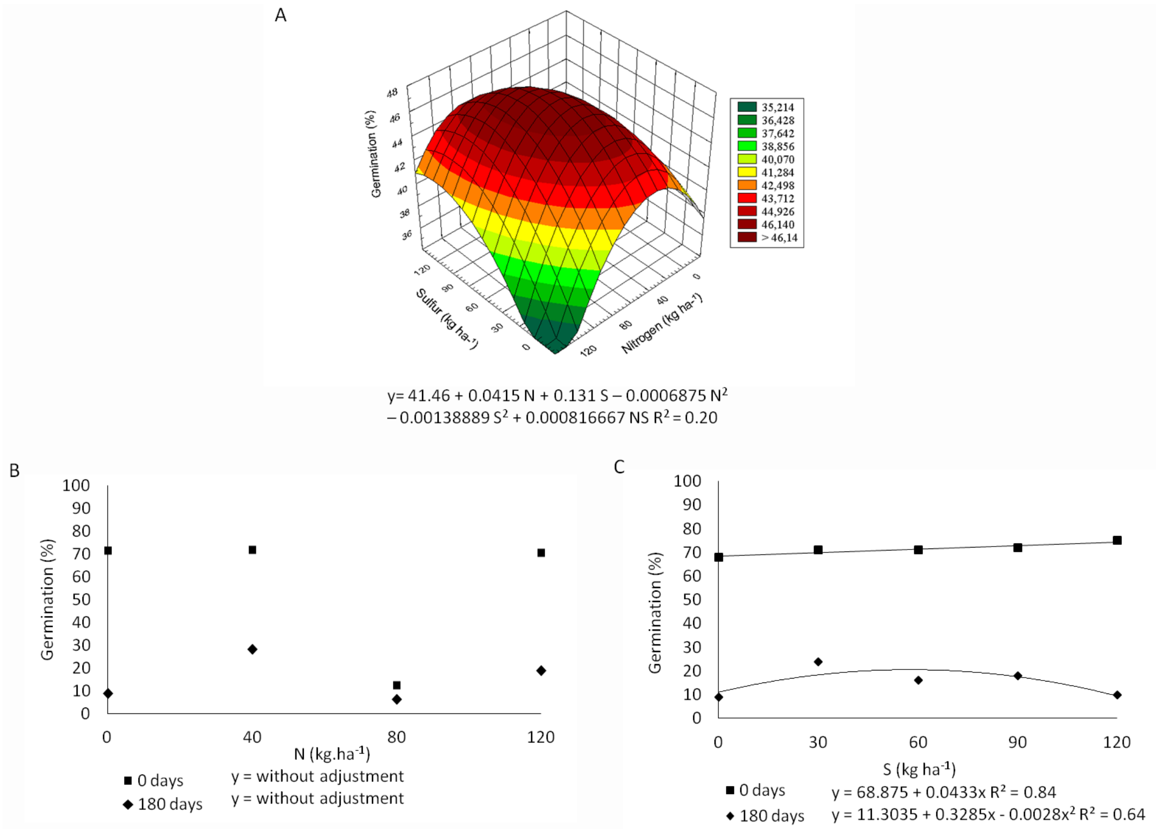


Figure 2. Germination (%) of oilseed rape seeds produced with different doses of nitrogen and sulfur (A). Germination (%) of oilseed rape seeds produced with different doses of nitrogen and stored for 180 days (B). Germination (%) of oilseed rape seeds produced with different doses of sulfur and stored (C).

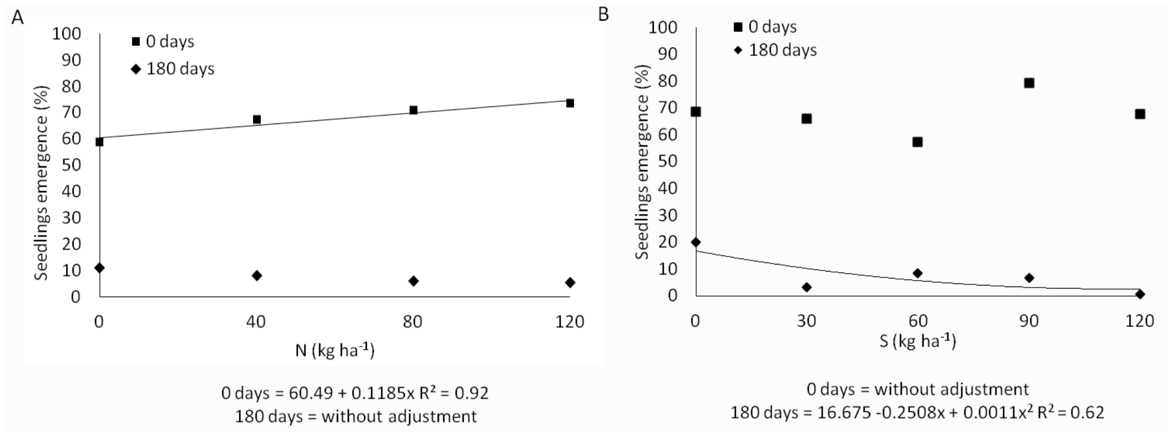


Figure 3. Seedlings emergence (%) of oilseed rape seedlings produced with different doses of nitrogen and stored for 180 days (A). Seedlings emergence (%) of oilseed rape seedlings produced with different doses of sulfur and stored (B).

With regard to the accelerated aging of seeds, there was a significant effect ($p < 0.05$) of the interaction between the nutrients (N × S) and there was a significant adjustment of the response surface for the interaction of factors (Figure 4A), which indicates that N restriction during production had a negative impact on the physiological potential of the seeds, even when increasing doses of S were applied alone. On the other hand, some authors have reported that, under conditions of S deficiency, oilseed rape seeds exhibit an increased number of abnormal seedlings (Brunel-Muguet et al*.,* 2015a), and that oilseed rape seeds with lower S content exhibited lower germination rates, which suggests the formation of dormant seeds under these conditions (Eggert and von Wirén, 2013; D'Hooghe et al., 2014). Germination of the seeds produced is a crucial trait for crop establishment and, consequently, for grain production (Brunes et al*.,* 2015). The results obtained in the present study indicated that the application of N has positive effects on germination, but this depends on the application of S to a certain extent.

There was a significant interaction ($p < 0.05$) between doses of N and storage, and a significant regression fit was only observed in the assessment of the effect of N on the vigor of the recently harvested seeds by the accelerated aging (maximum result: 32% with 37 $kg.ha^{-1}$ N) (Figure 4B); for the stored seeds, the minimum result was 18% with the 110 $kg.ha^{-1}$ S (Figure 4C). These results showed that storage was detrimental to the physiological potential of the oilseed rape seeds, even under controlled conditions.

There were significant effects of the N × S and N × storage interactions ($p < 0.05$) on the dry mass of the aerial parts and there was a significant adjustment of the response surface for the interaction of factors (Figure 5); the maximum aerial dry mass was 0.090 g at the 40 $kg.ha^{-1}$ dose of N associated with the 66 $kg.ha^{-1}$ dose of S (Figure 5), which

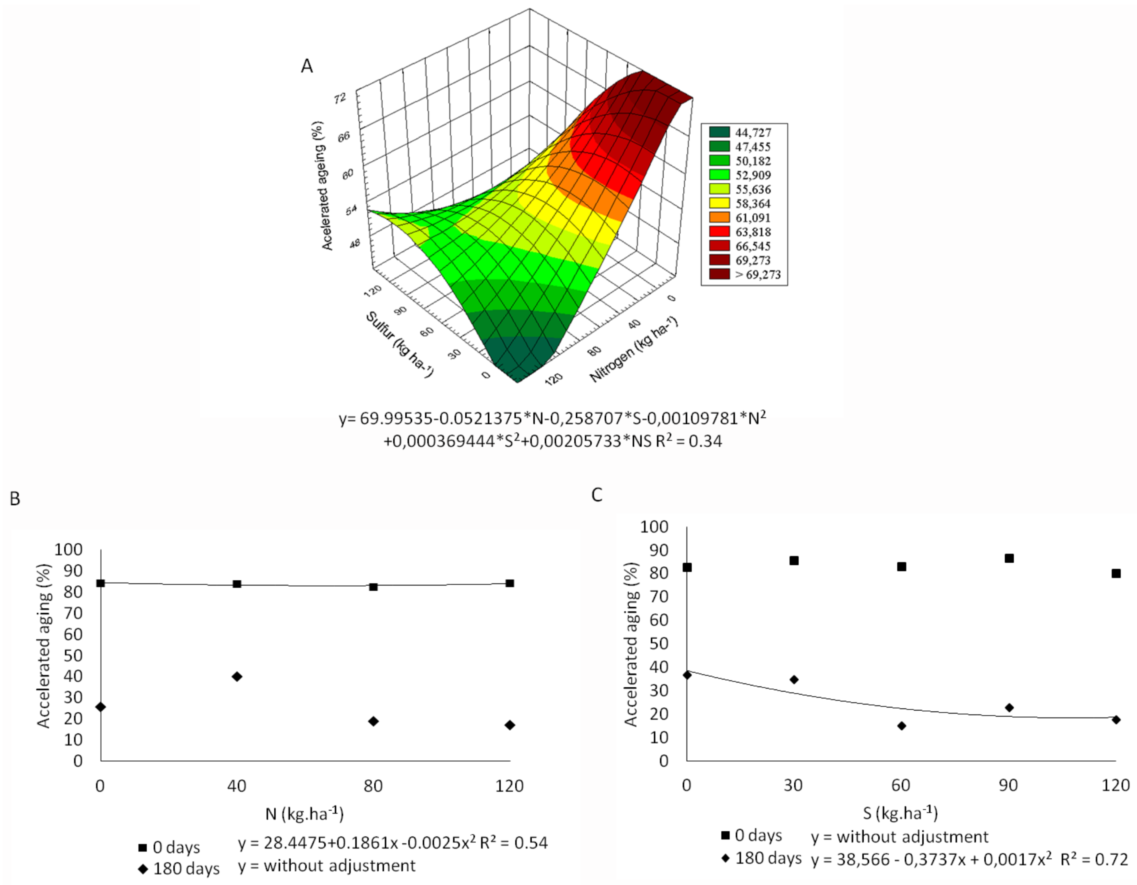
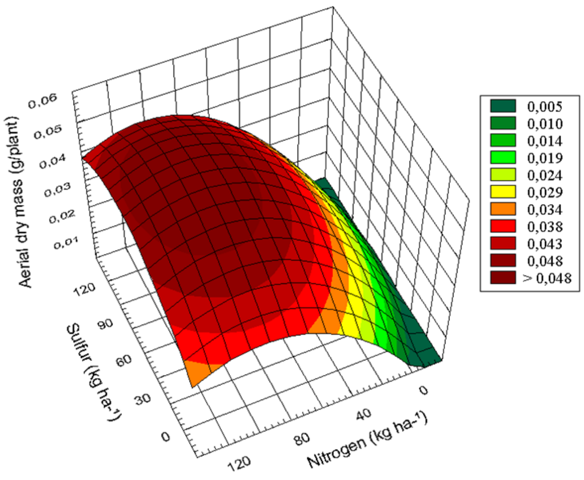


Figure 4. Accelerated aging (%) of oilseed rape seeds produced with different doses of nitrogen and sulfur (A). Accelerated aging (%) of oilseed rape seeds produced with different doses of nitrogen and stored (B). Accelerated aging (%) of oilseed rape seeds produced with different doses of sulfur and stored (C).



$$y = 0.00786625 + 0.000271969\ N + 0.000706\ S - 0.00000202734\ N^2 - 0.000003611\ S^2 + 0.000000191667\ NS\quad R^2 = 0.36$$

Figure 5. Aerial dry mass (g) of oilseed rape seeds produced with different doses of nitrogen and sulfur.

indicated that, at these doses, fertilization with N and S was effective in promoting the translocation of reserves from the embryo for the growth of the aerial part of the plant. According to Kopriva et al. (2016), oilseed rape has the highest demand for S of all crops; however, in the present study the benefits of supplying S for the formation of vigorous seeds appeared to be more strongly associated with the supply of N, based on the results of germination and vigor by the accelerated aging and assessments of field emergence and seedling performance.

There was no significant effect of the interaction between doses of N and S ($p > 0.05$) on root dry mass, or on the length of the aerial part, with the mean results for these parameters being 0.1248 g, 0.078 g, and 6.78 cm, respectively. There was a significant effect of the N × S interaction on root length and there was a significant adjustment of the response surface for the interaction of factors (Figure 6A), with minimum length of 4.5 cm observed for the combination of 0 $kg.ha^{-1}$ of N and 73.75 $kg.ha^{-1}$ of S and a maximum length of 6.15 cm observed for the combination of 120 $kg.ha^{-1}$ of N and 78.6 $kg.ha^{-1}$ of S (Figure 6A). These results showed the positive effects of the association between the nutrients on the formation of vigorous oilseed rape seeds. In addition, there was a significant effect of the interaction between nutrients and storage, and a significant linear regression was found only for the data for stored seeds, with the maximum root length being 5.88 cm at the 56.83 $kg.ha^{-1}$ dose of N (Figure 6B) and root length increasing as increasing S dose at intervals of 0.0054 (Figure 6C). These results indicated that, although storage reduces oilseed rape seed vigor, the application of the two nutrients had positive effects on the preservation of seed physiological potential, especially when the effect of S on root growth was assessed in isolation (Figure 6C).

Overall, the positive effects on seed germination and vigor observed for the application of S associated with N at the 120 $kg.ha^{-1}$ dose of N and are in line with the findings of Narits (2010) in Jõgeva, Estonia, who reported that oilseed rape seeds had higher oil content (2113 $kg.ha^{-1}$) with an N dose of 120 $kg.ha^{-1}$ divided among three applications. In a previous study conducted with the Hyola 61 hybrid in Dourados (MS), Sanches et al. (2014) also observed an increase in seed weight and oil content with increasing doses of N up to 90 $kg.ha^{-1}$. However, according to Kaefer et al. (2014) oilseed rape in Brazil is not as responsive to the use of N as it is in other countries, where yields reach 225 $kg.ha^{-1}$. This is probably because of the high temperatures that are common during the reproductive stage of the crop in tropical regions.

The results obtained herein indicate that there are contrasting effects of combined fertilization with N and S on oilseed rape seed germination and vigor, and these effects should be considered jointly with regard to seed storage conditions. In this study, the absence of N caused changes in seed physiological potential that limited the expected effects of increased S availability during seed production.

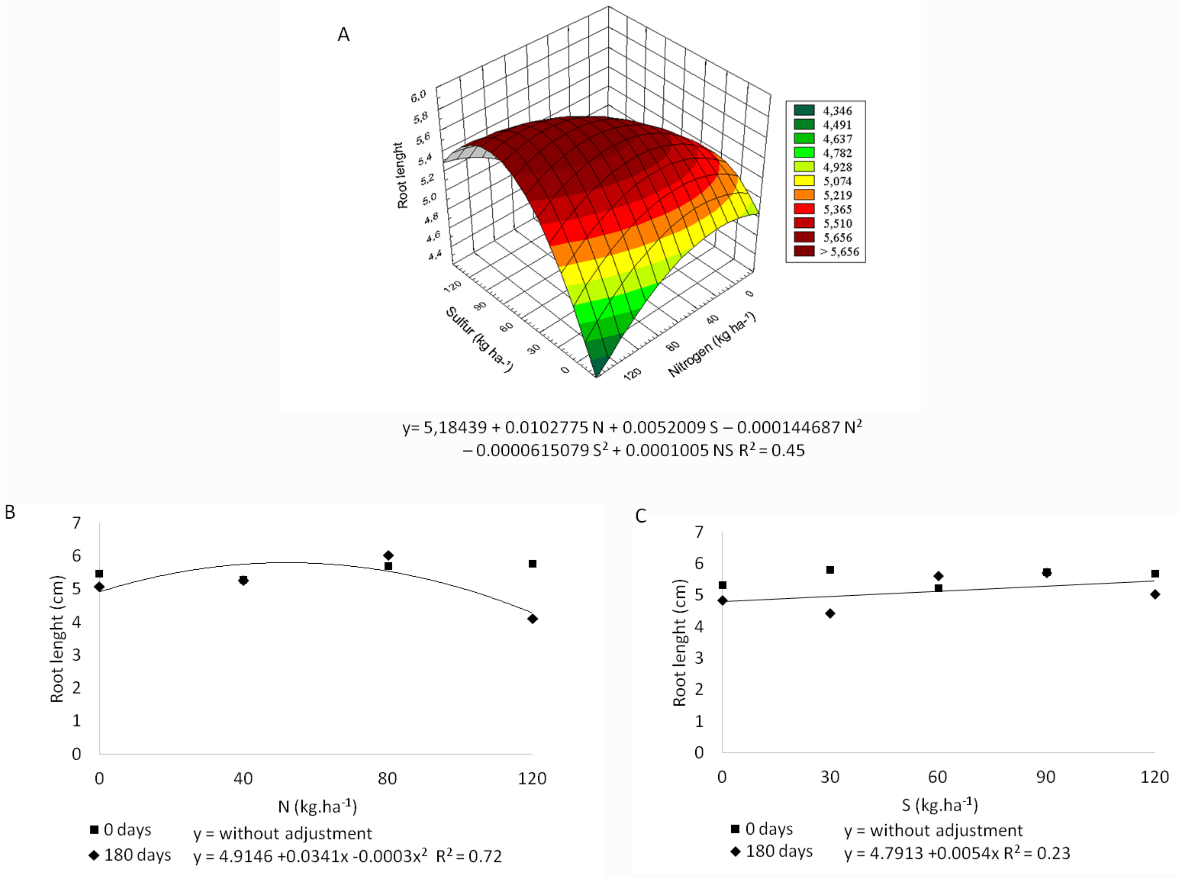


Figure 6. Root length (RL) (cm) of oilseed rape seedlings produced with different doses of nitrogen and sulfur (A). Root length (RL) (cm) of oilseed rape seedlings produced with different doses of nitrogen and stored (B). Root length (RL) (cm) of rapeseeds produced with different doses of sulfur and stored (C).

CONCLUSIONS

Fertilization with N and S may be effective to ensure optimal oilseed rape crop performance since the combination of doses of N and S had a positive effect on the physiological quality of oilseed rape seeds.

The application of these nutrients promoted the formation of seeds with higher germination and vigor as assessed by the accelerated aging, field emergence, dry mass of the aerial part and root growth. Seed storage for a period of 180 days had a negative effect on the physiological quality of the oilseed rape seeds.

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