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NITROGEN RATES AND SIDE-DRESSING TIMING ON SWEET CORN SEED PRODUCTION AND PHYSIOLOGICAL POTENTIAL[1]

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ABSTRACT – Sweet corn is an important crop because of its seeds with high total sugar and low starch contents. As common corn, this group requires an adequate amount of nitrogen to reach high yields. However, the studies on nitrogen and sweet corn are performed for ear yield instead of seed yield. As seeds are the main propagation method for this species, we proposed to evaluate the effects of nitrogen rates as side-dressing at different plant stages of a sweet corn seed production. Sweet corn seeds (variety BR 400) were sown in Latosol (Oxisol), and a 3×2+1 factorial scheme was designed with three nitrogen rates (40, 80, and 120 kg ha^{-1}) at two plant stages (V_6 and R_1) plus the control (no nitrogen side-dressing). The evaluated variables were seed yield, protein content, P and Zn contents, germination, and vigor rates. We concluded that nitrogen applied at a rate of 120 kg ha^{-1} at V_6 increases seed yield and maintains unaltered the protein content in seeds of sweet corn (BR 400 variety). Neither germination nor seed vigor increases when nitrogen rates are increased or administered at different stages of plant development. We also noted a slight decrease in P content or an increase in Zn content of seeds at low nitrogen rates; however, they are insufficient to promote changes in the physiological potential of sweet corn seeds.

Keywords: *Zea mays*. Yield. Protein content. Germination. Vigor.

DOSES DE NITROGÊNIO E ÉPOCA DE COBERTURA PARA PRODUÇÃO E POTENCIAL FISIOLÓGICO DE SEMENTES EM MILHO DOCE

RESUMO - O milho doce é uma cultura importante por causa do seu tipo de sementes com índice elevado dos açúcares totais e baixo índice do amido. Como o milho comum, este necessita de quantidade adequada de nitrogênio para atingir altas produtividades, no entanto, os estudos que envolvem nitrogênio e milho doce são realizados para o rendimento das espigas e não para a produção de sementes. Como a semente é o principal método de propagação dessa espécie, foi avaliado os efeitos das doses de nitrogênio em cobertura em diferentes estádios fenológicos para produção e potencial fisiológico de sementes de milho doce. As sementes de milho doce (variedade BR 400) foram semeadas em Latossolo e um esquema fatorial de tratamento 3 × 2 + 1 foi instalado com três doses de nitrogênio 40, 80 e 120 kg ha^{-1} e dois estádios fenológicos (V_6 e R_1) mais o controle (sem nitrogênio em cobertura). Foram avaliadas a produtividade de sementes, teores de proteína, P e Zn, germinação e vigor. Como conclusão, o nitrogênio fornecido em V_6 a 120 kg ha^{-1} aumenta a produtividade de sementes e mantém os teores de proteína inalterados. A germinação da semente e o vigor não aumentam quando as taxas de nitrogênio são acrescidas ou fornecidas em diferentes estádios fenológicos. Diminuição ligeira do teor de P ou o aumento do teor de Zn nas sementes são observados a uma dose baixa de nitrogênio entretanto não suficientes para promover alterações no potencial fisiológico das sementes do milho doce.

Palavras-chave: *Zea mays*. Produtividade. Teor de proteína. Germinação. Vigor.

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INTRODUCTION

Sweet corn (*Zea mays* L.), Saccharata group, is a plant with potential to contribute to energy generation by plant biomass (BARROS-RIOS et al., 2015) and can produce seeds with high total sugar and low starch contents, enhancing its quality for human consumption, both *in natura* or processed (KWIATKOWSKI; CLEMENTE; SCAPIM, 2011). When compared to common corn, this group has mutant alleles blocking sugar conversion to starch, increasing the total contents in seeds (JHA; SINGH; AGRAWAL, 2016).

For *Z. mays* plants, nitrogen is an important macronutrient for being one of the most accumulated in plants during their development, only requiring a supplementary addition via side-dressing to reach high yields (OKUMURA et al., 2014). Corn plants with a high level of nitrogen increase leaf sugar concentration, dry weight, photosynthesis rate and CO_2 assimilation, resulting in adequate physiological status (JIN et al., 2015). Also, some studies have reported an increase in grain protein contents by applying high levels of nitrogen either as side-dressing at vegetative stage or at late stages (at silking), improving corn nutritional quality (SILVA et al., 2005; SHARIFI; NAMVAR, 2016).

Protein accumulation in seeds is important because they represent the first source of amino acids within the first hours of imbibition, as free amino acids in dry seeds are insufficient for protein synthesis (BEWLEY et al., 2013). In *Z. mays*, the accumulation of storage proteins occurs during the early stages of endosperm development, being retained until later stages in the endoplasmic reticulum, protein bodies, or in aleurone cells (REYES et al., 2011). Therefore, the high protein content in seeds can represent a beneficial balance of amino acids in seeds, enhancing seed development speed and uniformity.

Besides protein content, nitrogen has the potential to affect the balance of other nutrients, such as phosphorus and zinc content (MALAVOLTA; VITTI; OLIVEIRA, 1997). Both nutrients (P and Zn) are essential for seedling establishment and initial growth. As suggested by White and Veneklaas (2012), seed P content is the only source of this mineral available for the initial growth of seedlings. Additionally, Boonchuay et al. (2013) observed a progressive increase in seedling weight with Zn increment in seeds of rice.

Although there are studies on nitrogen management for common corn (LEAL et al., 2013; VALDERRAMA et al., 2014), little is known about sweet corn (Saccharata group), which has seeds with different properties; hence, more results on the optimal rate and plant stage for nitrogen application are missing. According to Cruz et al. (2015), nitrogen as a side-dressing in sweet corn increase the number of commercial ears; however, these authors made no relationship between it and seed yield and nutritional aspect of seeds, such as protein accumulation, nutritional balance, and seed physiological potential.

Thus, there is little information when the subject is nitrogen management and sweet corn seed production, mainly concerning seed yield, protein content, nutritional balance, and physiological potential. Based on the hypothesis that the nitrogen can improve seed yield, protein content, phosphorus/ zinc contents, and seed physiological potential, we proposed to evaluate the effects of nitrogen rates as side-dressing applied at different plant stages in sweet corn plants.

MATERIAL AND METHODS

Sweet corn seeds (variety BR 400, super sweet brittle 1) were sown in an area belonging to the State University of Londrina. The local soil is classified as first level by the IUSS working group WRB (2015), being a Ferralsol. Local weather is classified by Köppen-Geiger's classification as Cfa (temperate without dry season and hot summers). The area was previously grown with wheat and the soil chemical properties determined within 0.0-0.2 m depth. The analysis results are as follow: pH ($CaCl_2$) = 5.00, H + Al = 3.42 $cmol_c\ dm^{-3}$, Ca = 9.77 $cmol_c\ dm^{-3}$, Mg = 1.76 $cmol_c\ dm^{-3}$; K = 0.84 $cmol_c\ dm^{-3}$, P = 14.49 mg dm^{-3}, and organic matter = 28.60 g dm^{-3}. Based on Oliveira (2003), the sowing fertilization rates were 30 kg ha^{-1} N, 40 kg ha^{-1} P_2O_5, and 40 kg ha^{-1} K_2O.

Seed sowing was made at the rate of 7 seeds per row meter spaced in 0.9 m to reach the plant population recommend for BR 400 (70,000 plants/ha). The growing was carried out during spring, as shown in Figure 1.

The study was performed in a randomized complete block design, in a 3×2+1 factorial scheme with four replications. Treatments consisted of a combination of three (3) nitrogen rates applied as side-dressing (40, 80, and 120 kg ha^{-1}) at two (2) plant stages (V_6 stage: at least 50% plants with six leaves; R_1 stage – silking: at least 75% plants showing ears with visible stigmas) plus a control (no nitrogen application). The experimental unit area (plot) was 18m^2 (5.0m×3.6m), and the useful area for yield estimation was 7.2 m^2 (4.0m×1.8m). The nitrogen source used on side-dressing was urea CO $(NH_2)_2$, and plant development stages identified as proposed by Ritchie, Hanway and Benson (1993). During plant development, soil moisture, weeds, diseases, and pest were controlled as recommended to guarantee a great plant development. When seeds reached 20% moisture, ears were harvested and seeds dried until 13%.

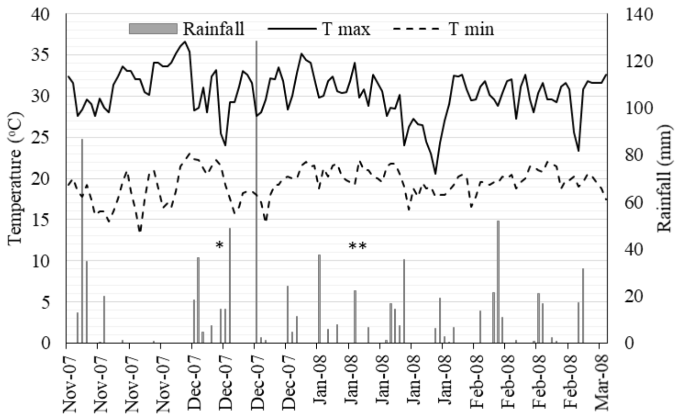

Figure 1. Weather data during spring season on the campus of the State University of Londrina, located at 51°11'E, 23°23'S, and 560 m above sea level (*N fertilization at vegetative stage; **N fertilization at reproductive stage).

The analyzed parameters followed the following procedures: *Seed yield* – determined for seeds with 13% moisture and harvested from the useful area. *Germination* – 50 seeds from each treatment were sown on 3 sheets of towel paper and kept at 25 ºC for seven days (single counting); the number of normal seedlings was determined as proposed by Rules for Seed Testing (BRASIL, 2009) with results expressed in percentage. *Accelerated aging* – 50 seeds from each treatment were placed on a stainless screen inside closed boxes (0.03 × 0.03 × 0.11 m) filled with 40 mL distilled water. The set was kept at 42 ºC for 72 h, according to Santos et al. (2002); then, the germination testing was performed as previously described. *Protein content* – seed samples from each treatment were dried in an oven (65 ºC) until obtaining constant mass. Afterward, total nitrogen was determined for each seed sample according to Malavolta, Vitti and Oliveira (1997), and the results expressed as proposed in the Kjeldahl method where protein content is estimated based on total nitrogen content × 6.25 (conversion factor). P and Zn were analyzed by acid digestion with a nitropercloric mixture in a block digester, being analyzed by spectrophotometry, as proposed by Malavolta, Vitti and Oliveira (1997).

Data analysis was performed with a complex treatment structure using multiple contrast tests, according to Schaarschmidt and Vaas (2009). In this analysis, contrasts are determined to perform comparisons between the most interesting variables (Table 1). The contrasts consisted of the null hypothesis (H_0): **A**: there is no difference between control and any nitrogen rate (contrasts 1 to 6); **B**: there is no difference among nitrogen rates applied at V_6 stage (contrasts 7 to 9); **C**: there is no difference among nitrogen rates applied at R_1 stage (contrasts 10 to 12); **D**: there is no difference among nitrogen rates applied at V_6 stage or at R_1 stage (contrasts 13 to 15).

RESULTS AND DISCUSSION

In general, treatments showed low seed yields because the study was carried out with the variety BR 400 (Table 2). It is an open-pollinated variety still important for small farmers and has good acceptability but low yield compared with hybrids. The environmental conditions were good for plant development with a total rainfall of 853 mm (well distributed) and mean temperature of 24.9 ºC. According to Albuquerque (2010), corn plant requires from 380 mm to 550 mm water to complete the cycle.

Seed P contents were slightly higher than those observed by White and Veneklass (2012) in common corn. These authors stated that 75.6% of the total P in seeds is in phytin compounds, which make an important source of P during plant initial development. Seed Zn content was slightly higher than that observed by Jin et al. (2013). For Alscher, Erturk and Health (2002), zinc is a constituent of an isoform of superoxide dismutase, an enzyme able to transform the superoxide radical into hydrogen peroxide.

For seed physiological potential (Table 2), we observed low normal seedlings evaluated by germination and accelerated aging tests, what is normal for this group of *Zea mays*. According to Wilson-Junior and Mohan (1998), for sweet corn sh-2, the main problem for seed vigor is the extreme levels of sucrose, which results in an embryo sugar imbalance and membrane disruption, producing seed lots with poor vigor. In addition, we observed a slight increase in accelerated aging results. It could have occurred because of a thermic and humid treatment, which increases the number of normal seedlings by decreasing germination reducer microorganisms or by promoting similar effects of seed priming.

Table 1. Contrast coefficient of the 15 comparisons proposed to perform this study.

Contrast number[1]	Comparison	Contrast Coefficient						
		Control	V_6			R_1		
		---------------- kg N ha^{-1} ----------------						
		0	40	80	120	40	80	120
1	Control × 40 kg N ha^{-1} (V_6)	1	-1	0	0	0	0	0
2	Control × 80 kg N ha^{-1} (V_6)	1	0	-1	0	0	0	0
3	Control × 120 kg N ha^{-1} (V_6)	1	0	0	-1	0	0	0
4	Control × 40 kg N ha^{-1} (R_1)	1	0	0	0	-1	0	0
5	Control × 80 kg N ha^{-1} (R_1)	1	0	0	0	0	-1	0
6	Control × 120 kg N ha^{-1} (R_1)	1	0	0	0	0	0	-1
7	40 kg N ha^{-1} × 80 kg N ha^{-1} (V_6)	0	1	-1	0	0	0	0
8	40 kg N ha^{-1} × 120 kg N ha^{-1} (V_6)	0	1	0	-1	0	0	0
9	80 kg N ha^{-1} × 120 kg N ha^{-1} (V_6)	0	0	1	-1	0	0	0
10	40 kg N ha^{-1} × 80 kg N ha^{-1} (R_1)	0	0	0	0	1	-1	0
11	40 kg N ha^{-1} × 120 kg N ha^{-1} (R_1)	0	0	0	0	1	0	-1
12	80 kg N ha^{-1} × 120 kg N ha^{-1} (R_1)	0	0	0	0	0	1	-1
13	40 kg N ha^{-1} (V_6) × 40 kg N ha^{-1} (R_1)	0	1	0	0	-1	0	0
14	80 kg N ha^{-1} (V_6) × 80 kg N ha^{-1} (R_1)	0	0	1	0	0	-1	0
15	120 kg N ha^{-1} (V_6) × 120 kg N ha^{-1} (R_1)	0	0	0	1	0	0	-1

[1]Data analysis was performed through Proc GLIMMIX, in SAS University Edition®.

Table 2. Averages of seed yield, phosphorus and zinc contents, germination, and accelerated aging in sweet corn.

Variety	Parameter	Average	Coefficient of variance
BR 400	Yield	2234 kg/ha	10.5%
	Seed P content	5.3 g/kg	13.1%
	Seed Zn content	30.91 mg/kg	11.3%
	Germination	75 %	7.8%
	Accelerated aging	83 %	10.5%

For contrasts 1 to 6 (Hypothesis **A**), we observed a yield increment for 120 kg N ha^{-1} at V_6 stage. With 95% confidence, nearly 287 kg ha^{-1} increment in seed yield is expected under this rate and at this vegetative stage, showing the benefits brought by nitrogen as side-dressing in sweet corn. This result was confirmed with the ones observed for contrasts 7 to 9 (Hypothesis **B**), which identified a significant increment in seed yield when 120 kg N ha^{-1} nitrogen was provided at V_6 (Table 1 and Figure 2). When nitrogen was applied, at any rate, as side-dressing in the reproductive stage R_1 (contrast 10 to 12 of Hypothesis **C**), there was no increment in seed yield; the application of 120 kg N ha^{-1} at R_1 decreased seed yield in 267 kg ha^{-1}; therefore, we concluded no application should be performed during this stage.

The optimal nitrogen rate in sweet corn is variable since there are many responsive varieties and hybrids, which show different yields. In this study, we used a variety (BR 400) with low yield potential compared to hybrids but still important for low-income farmers, unable to buy hybrid seeds. There is no specific nitrogen recommendation for sweet corn; however, we observed that 80 kg N ha^{-1} is suboptimal to improve seed yield and an increment was only reached for 120 kg N ha^{-1} N at V_6. A similar outcome was reported by Okumura et al. (2014), who found a rate of 110.84 kg N ha^{-1} as optimal for sweet corn ear production.

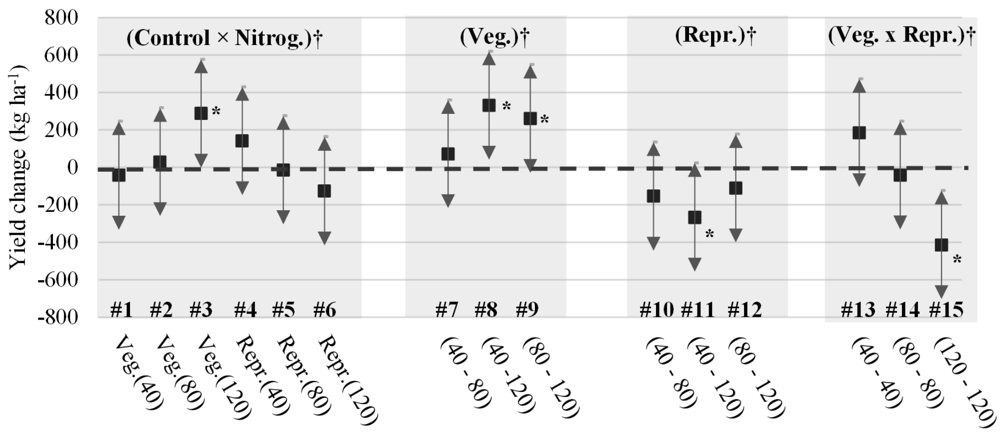

Figure 2. Increase/ decrease in seed yield expressed in simultaneous 95% confidence intervals for 15 contrasts (Table 1). Y-axis expresses the comparisons between control and each nitrogen rate (contrasts 1 to 6), among nitrogen rates at vegetative stage (contrasts 7 to 9), among nitrogen rates at reproductive stage (contrasts 10 to 12) and between each nitrogen rate at vegetative and reproductive (contrasts 13 to 15). † At least one significant contrast; *significant contrast at 5%.

Seed protein content had no alteration for any tested contrast between nitrogen rates or plant stages in sweet corn (Figure 3). One hypothesis for this result is regarding the effect of genetic and environment interaction on this characteristic as Silva et al. (2005) observed different potential to accumulate crude protein in grains.

Seed P contents changed as a function of nitrogen rate and application timing (Figure 4A). At the optimal nitrogen dressing rate for yield (120 kg N ha^{-1} in V_6), no increment was observed in P and Zn contents, therefore, resulting in an equilibrated nutritional status. Conversely, the lower nitrogen rates (40 and 80 kg N ha^{-1} in V_6) resulted in smaller seed P contents (contrasts 1 and 2). This outcome was reaffirmed in contrasts 8 and 9, where the rate of 120 kg N ha^{-1} resulted in higher seed P contents compared to the other rates (40 and 80 kg N ha^{-1} in V_6).

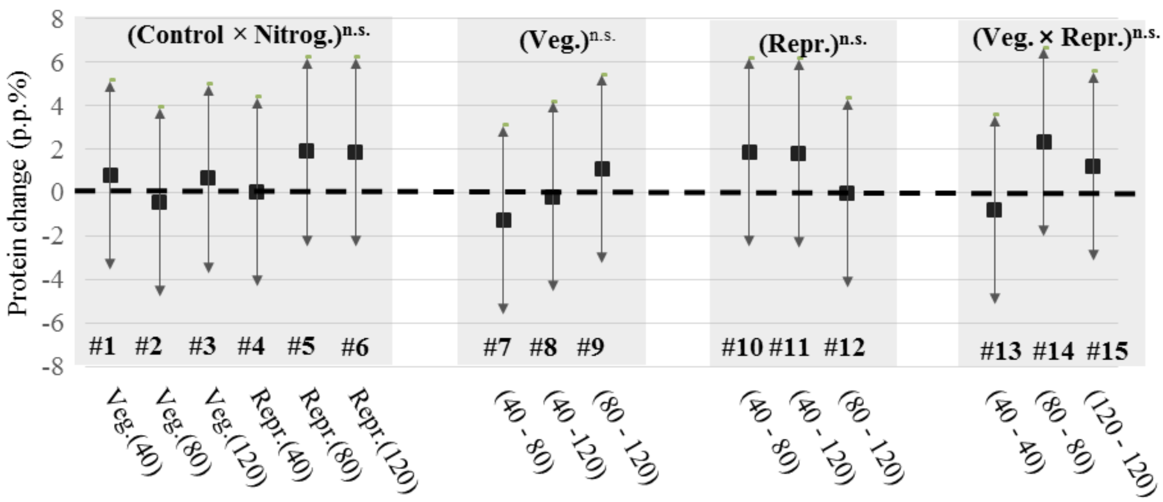

Figure 3. Increase/ decrease in protein content (percent point) expressed in simultaneous 95% confidence intervals for 15 contrasts (Table 1). Y-axis expresses the comparisons between control and each nitrogen rate (contrasts 1 to 6), among nitrogen rates at vegetative stage (contrasts 7 to 9), among nitrogen rates at reproductive stage (contrasts 10 to 12) and between each nitrogen rate at vegetative and reproductive (contrasts 13 to 15). † At least one significant contrast; *significant contrast at 5%.

The Zn content in seeds had no increment at the optimal nitrogen rate for yield (120 kg N ha^{-1}) (Figure 4B). In contrast, the lower rates (40 and 80 kg N ha^{-1} in V_6) promoted a slight increase in Zn contents (contrasts 1 and 2) for contrasts 8 and 9, showing Zn content reductions in seed when the optimal rate was applied. The nitrogen application during reproductive stage R_1 increased Zn seed contents at 80 and 120 kg N ha^{-1} (contrasts 5 and 6).

P and Zn are important for either seed germination or human nutrition. Moreover, P is a nutrient with benefits to seed germination because is part of the phytin molecule, which provides energy to start the germination process. On the other hand, P as part of the phytin molecule is nutritionally undesired since negatively charged phosphate groups can bind essential dietary minerals, such as zinc, calcium, and iron (BEWLEY et al., 2013).

For being a cofactor of many enzymes, when Zn is unavailable in the soil, the seed reserves are the main sources of this mineral for the first germination processes. Rengel and Graham (1995) observed a fast initial growth of wheat seedlings with high Zn contents if compared to those grown on a Zn-deficient soil. In this study, we concluded that even significant, these changes were insufficient to promote any alteration in seed quality.

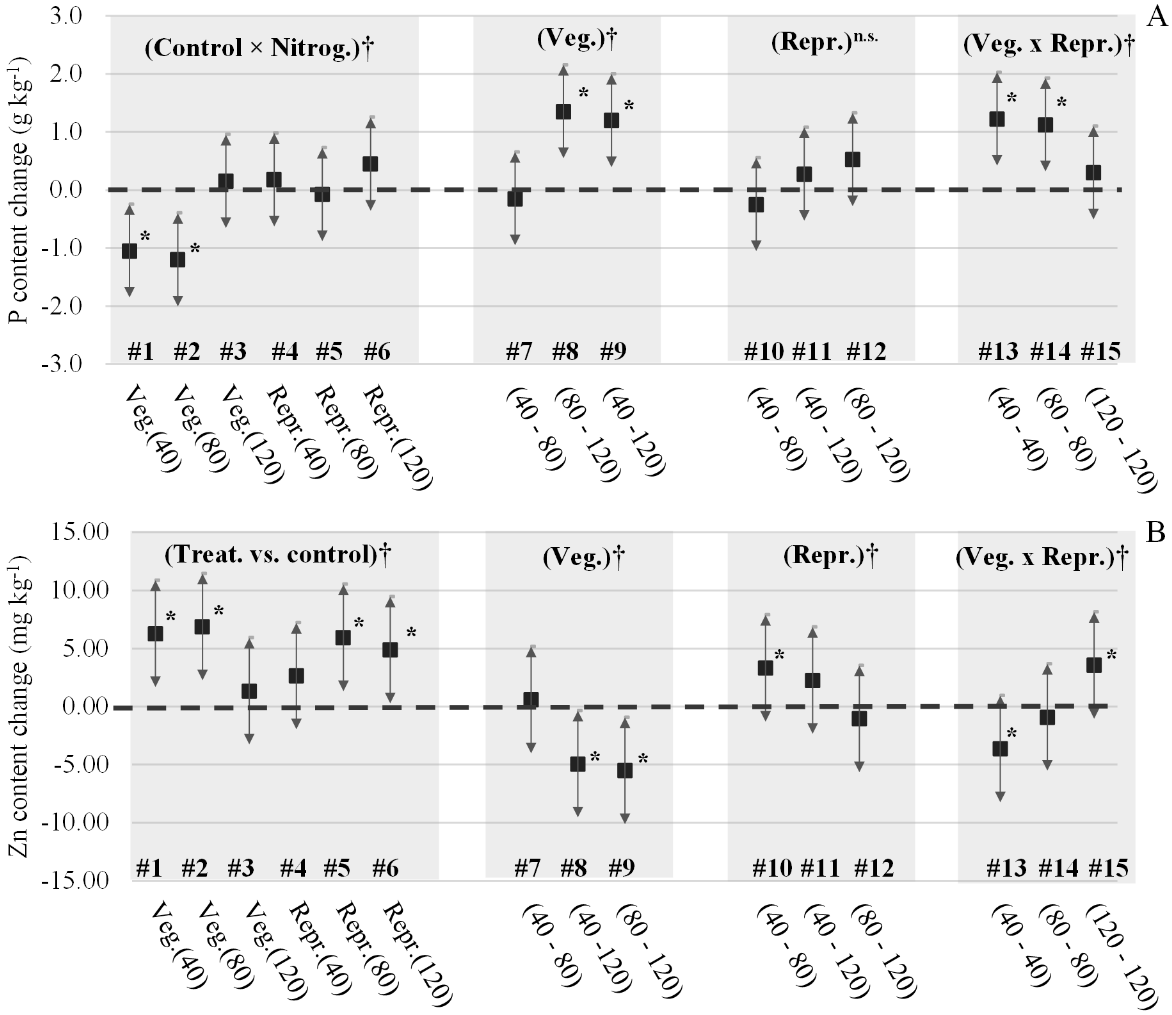

Figure 4. Phosphorus (A) and Zinc (B) contents in sweet corn seeds expressed in simultaneous 95% confidence intervals for 15 contrasts (Table 1). Y-axis expresses the comparisons between control and each nitrogen rate (contrasts 1 to 6), among nitrogen rates at vegetative stage (contrasts 7 to 9), among nitrogen rates at reproductive stage (contrasts 10 to 12) and between each nitrogen rate at vegetative and reproductive (contrasts 13 to 15). † At least one significant contrast; *significant contrast at 5%.

Concerning the physiological potential of seeds, neither rates nor the timing of nitrogen application had significant results (Figure 5A and 5B). Even though slight changes in P and Zn contents were registered (Figure 4A and 4B), corn seeds kept germination speed and vigor unaltered. On the one side, nitrogen has been related in the literature as an important nutrient to increase yield, on the other hand, for seed physiological potential, many studies have reported no relationships between nitrogen rates and seed germination/ vigor. Likewise, Zucareli et al. (2012) tested nitrogen rates in sweet corn and observed no increment in seed physiological potential, even at high rates.

In general, sweet corn had a good response to nitrogen rates, showing increments in seed yield and keeping seed physiological potential unaltered. Therefore, we recommend the application of nitrogen to produce sweet corn seeds with no germination and vigor damages.

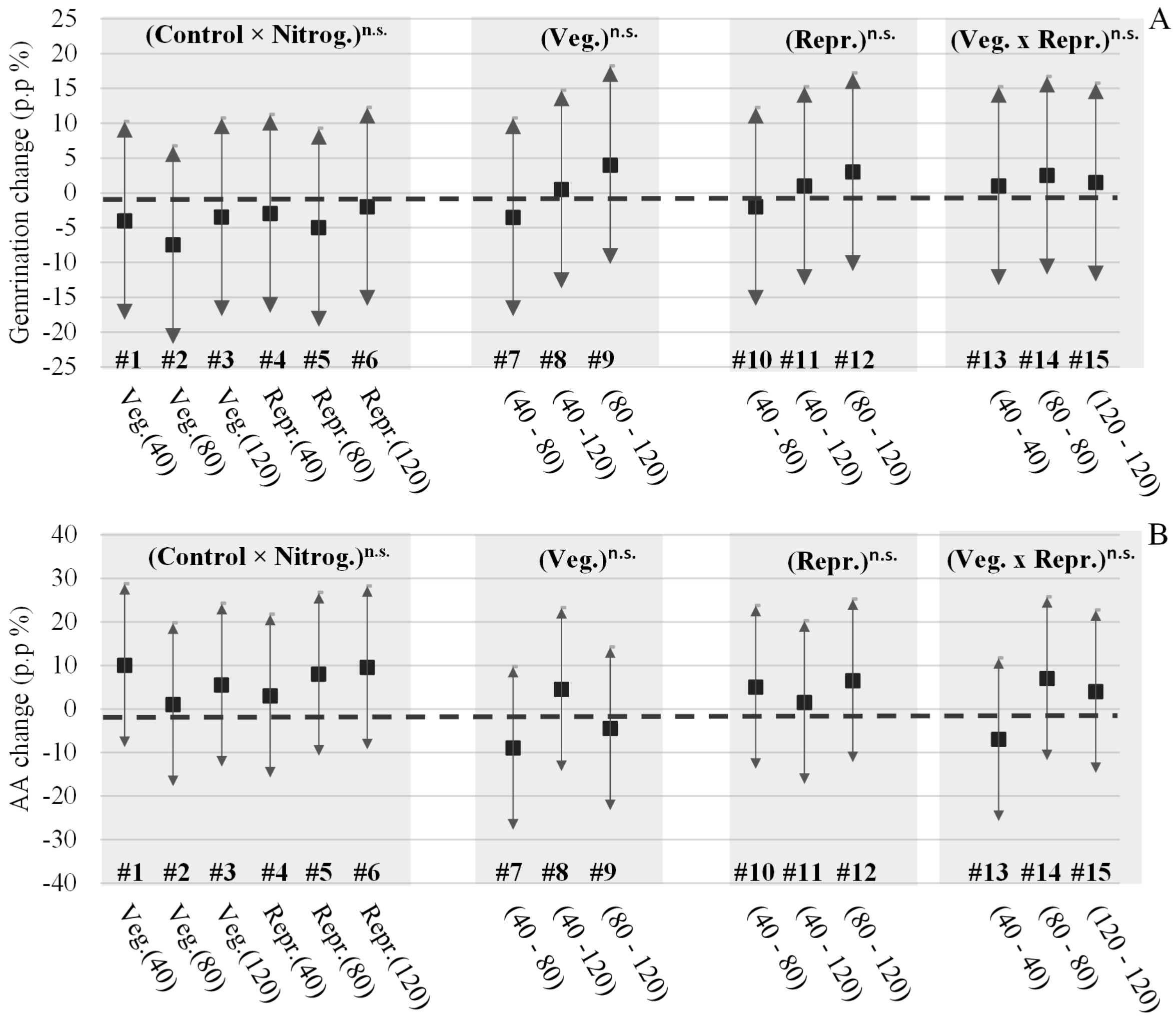

Figure 5. Variations in germination (A) and accelerated aging - AA (B) of sweet corn seeds (percent points) expressed in simultaneous 95% confidence intervals for 15 contrasts (Table 1). Y-axis expresses the comparisons between control and each nitrogen rate (contrasts 1 to 6), among nitrogen rates at vegetative stage (contrasts 7 to 9), among nitrogen rates at reproductive stage (contrasts 10 to 12) and between each nitrogen rate at vegetative and reproductive (contrasts 13 to 15). † At least one significant contrast; *significant contrast at 5%.

CONCLUSION

The application of 120 kg ha^{-1} nitrogen at V_6 increased seed yield and maintained the protein content unaltered in seeds of sweet corn BR 400 variety. Neither seed germination nor seed vigor increased with increasing nitrogen rates or application times. Smaller nitrogen rates promoted a slight decrease in P and increase in Zn contents in sweet corn seeds but insufficient to promote changes in their physiological potential.

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