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EFFECT OF NITROGEN FERTILIZATION ASSOCIATED WITH WATER-RETAINING GEL ON LETTUCE SEED QUALITY AT DIFFERENT MOISTURE LEVELS

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Abstract–The objective of this study was to evaluate the efficiency of nitrogen fertilization associated with water-retaining as to the quality of lettuce seeds at different moisture levels. The experimental design used was composed of randomized blocks, in a 2 x 5 + 1 factorial scheme, with two moisture levels (50 and 75% field capacity) and five doses of nitrogen (0, 25, 50, 100 and 200 kg ha^{-1}, with the addition of water-retaining gel, besides an additional treatment, without gel addition, with moisture at a level of 100% field capacity and standard nitrogen fertilization (100 kg ha^{-1}), which will consist of the control treatment, with five replications. The analyzed variables were: water content, germination, germination speed index, electrical conductivity, and accelerated aging. The evaluated factors showed no significant interaction, for most of the analyzed variables, with the exception of the germination speed index. For germination, there was significant effect for the 'levels of moisture in the substrate' factor. Comparing the means of the treatments with the control, for electrical conductivity, all treatments had lower EC values, which indicate better vigor. It is concluded that using water-retaining gel associated with nitrogen fertilization allows the use of lower levels of moisture in the substrate (50%), without affecting the quality of the produced seeds. N doses from 50 to 100 kg ha^{-1} with the use of water-retaining gel favors the production of seeds with greater vigor.

INTRODUCTION

Grown in all Brazilian regions, lettuce (*Lactuca sativa* L.) is a vegetable of great national prominence, appreciated for its flavor, nutritional quality and low cost (Resende *et al.,* 2007). In Brazil, there is a great diversity of lettuce cultivars available, and the main types of lettuce grown in order of economic importance are crisp, iceberg, butterhead and Romaine (Sala and Costa, 2012).

The crop is responsive to nitrogen fertilization, as it is basically composed of leaves and requires special nutritional management, mainly when the purpose of cultivation is seed production (Mota *et al.,* 2016). The physiological quality of its seeds responds differently to fertilization with N, due to the energy protein balance being dependent on the amount absorbed during the production cycle (Kolchinski and Schuch, 2004; Toledo *et al.,* 2007); however, there are no reports in the literature that guide the practice of nitrogen fertilization with evidence regarding the qualitative characteristics of crisp lettuce seeds.

For the good development of the crop, in addition to balanced nutritional management, the ideal maintenance of soil moisture is substantial, as a deficiency or excess of applied water, in addition to compromising plant development, causes other adverse effects to the production system. Some measures to increase this efficiency have been implemented, such as the application of water-retaining gels or polymers. Usually employed in perennial agriculture and forestry (Ferreira *et al.*, 2014), these molecules absorb and store rain or irrigation water, reducing the volume and frequency of necessary irrigation by 40 to 60%, maintaining moisture available for the plant gradually (Moraes, 2001).

Water-retaining gel reduces nutrient leaching, improves cation exchange capacity and increases water availability for plants (Vale *et al.*, 2006), and nitrogen is an extremely important nutrient in vegetable production. However, the study of the association of these factors and their effects on the production and physiological quality of lettuce seeds are scarce. In this sense, it is extremely important for agricultural production to know the outcome of combining them and thus assess the behavior of plants in seed production fields.

In this context, the objective was to evaluate the efficiency of nitrogen fertilization associated with water-retaining gel as to the physiological quality of lettuce seeds, at different moisture levels.

MATERIALS AND METHODS

The experiment was conducted in an acclimatized greenhouse and in the Seed Analysis Laboratory of the Department of Agrarian Sciences of the State University of Montes Claros, Janaúba Campus, Minas Gerais, Brazil. Crisp lettuce seeds, cultivar Veneranda, were used.

The experimental design used was composed of randomized blocks, in a 2 x 5 + 1 factorial scheme, with five replications. The treatments consisted of two levels of substrate moisture (50 and 75% of the substrate's retention capacity) and five doses of nitrogen (0, 25, 50, 100 and 200 kg ha^{-1}), with the addition of water-retaining gel (1g $plant^{-1}$), besides an additional treatment, without gel addition, with moisture at 100% field capacity, and standard nitrogen fertilization of 100 kg ha^{-1} (IAC, 2021) as the control. The water-retaining gel used in the present study is powdered and has neutral pH, density of 0.8 g dm^{-3}, granulometry of 3 to 5 mm, and absorption potential of 300 ml g^{-1}.

For seedling production, the seeds were sown in trays of expanded polyethylene (Styrofoam) with 128 cells, containing Bioplant® commercial substrate, and three seeds per cell. After germination and emergence, when the seedlings had the second definitive leaf, thinning was carried out, leaving only one seedling in each cell. Twenty-three days after sowing, when the seedlings had four definitive leaves, they were transplanted into plastic pots (7.5 liters) containing soil and sand in a 3:1 ratio, which were stored in the greenhouse. The average conditions of temperature and relative humidity recorded in the greenhouse, during planting until harvest, were 25 ± 3 ºC and 60%, respectively.

Planting fertilization, performed one week before the seedlings were transplanted, consisted of supplying 22 g of simple superphosphate and 10 g of potassium sulfate (Bernadino, 2016). Top-dressing fertilizations consisted of biweekly installments for nitrogen and potassium (urea and potassium sulfate), and the micronutrients were applied in top-dressing and in installments, at 30, 60 and 90 days. The applications followed the dosages of 60 mg dm^{-3} of potassium, 03 mg dm^{-3} of boron, 06 mg dm^{-3} of zinc, and 03 mg dm^{-3} of copper (Oliveira *et al.*, 1991). The top-dressing fertilizations with the nitrogen doses were carried out in installments, applied every two weeks after transplanting, using fertilizers that contained nitrogen, interspersing the sources at each application at the pre-established doses.

Irrigation was performed manually with the aid of a graduated cylinder, once a day in the late afternoon, in order to keep moisture at around 50, 75 and 100% of the substrate's retention capacity, in accordance with the treatments. To maintain the moisture levels, the pots were weighed to calculate the volume of water that needed to be applied, in order to maintain the pre-established moisture levels. The preventive control of pests and diseases was carried out in a conventional manner. To control silver leaf whiteflies (*Bemisia tabaci*), the TIGER 100 EC insecticide was used. Yellow adhesive traps were set up for pest monitoring and control.

To harvest the seeds, the inflorescences were manually cut when they had 70% of white plumage, which occurred 103 days after transplanting. Afterwards, the inflorescences were placed in plastic bags, which were sealed and taken to the laboratory, where said inflorescences were manually extracted and processed for quality evaluation.The water content of the seeds was determined by the oven method, at 105 ± 3 ºC for 24 h, using four replications of 50 seeds, with the results being expressed as percentage (Brasil, 2009).

The germination test was run using four replications of 50 seeds distributed on two sheets of Germitest® paper, previously moistened with distilled water in a volume equivalent to 2.5 times their dry weight, and placed in plastic gerboxes. The boxes containing the seeds were stored in a germinator regulated at a temperature of 20 ºC and constant light. The evaluations were performed on the seventh day after sowing, and the results were expressed as percentage of normal seedlings. Seedlings presenting essential structures (root system and aerial part) that were developed, proportional and healthy were considered normal (Brasil, 2009).

The germination speed index (GSI) was determined from daily counts of the number of normal seedlings until the seventh day after sowing. At the end of the test, the GSI was calculated, using the formula proposed by Maguire (1962). The electrical conductivity test was carried out with four replications of 50 seeds per treatment, with known mass, which were placed in plastic cups, with a capacity of 200 mL, containing 75 mL of distilled water. The cups containing the seeds were kept in a BOD chamber regulated at a temperature of 25 ºC for 24h. The solution was analyzed using a Digimed conductivity meter, model CD-21, and the results were expressed in $\mu S\ cm^{-1}\ g^{-1}$ of seeds, dividing the reading by the mass of the seeds (Vieira and Krzyzanowski, 1999).

The accelerated aging test was conducted using approximately 250 seeds for each treatment, uniformly distributed on an aluminum screen coupled to the inside of the gerboxes, which contained 40 mL of distilled water at the bottom. The boxes were covered and kept in a BOD-type chamber at 41 °C for 72 hours (Panobianco and Marcos Filho, 2001). Then, the seeds were subjected to the germination test, as described above, with evaluations of number of normal seedlings being performed four days after sowing.

The data were subjected to analysis of variance. The means for the moisture levels were studied through the F test, and the nitrogen doses, through regression analysis ($p<0.05$). The estimates of the regression parameters were evaluated by the "t" test ($p<0.05$). In addition to significance, the highest-degree models with biological behavior were chosen to explain the phenomenon. To verify the effect of the treatments in relation to the control, Dunnett's test was performed ($p<0.05$). Statistical analyses were conducted with the aid of the Statistical Software R: A Language and Environment for Statistical Computing (2012) and ASSISTAT 7.7.

RESULTS AND DISCUSSION

There was significant effect as to the interaction between moisture levels and nitrogen doses only for the germination speed index (GSI). Germination was influenced only by moisture levels. For the water content, electrical conductivity and accelerated aging variables, there was no significant effect as to any of the studied factors.

The results for the water contents of the seeds in the studied treatments varied from 7.3 to 10.2% of moisture, values considered ideal for orthodox seeds, which are able to tolerate drying at low moisture levels, such as lettuce (Costa, 2012), indicating that water content did not affect the results of the performed tests. If the seed reaches a water content above 12% moisture, its respiratory activity may become intense, which leads to a considerable consumption of reserve material and a decrease in energy, causing deterioration and loss of vigor (Carvalho and Nakagawa, 2012).

Regarding germination (Table 1), it is noted that the seeds produced in a substrate moistened with 50% retention capacity showed higher percentages of germination in relation to those produced in a substrate with greater availability of water (75% moisture). Possibly, the use of water-retaining gel associated with the reduction in moisture to 50% retention capacity proved to be favorable, promoting greater efficiency in the use of water for seed germination. Thus, by applying water-retaining gel, it is possible to use a smaller volume of water to produce seeds, without compromising their germination capacity.

Table 1. Germination (GER) of lettuce seeds grown under different levels of moisture in the substrate and as a function of using water-retaining gel

Moisture (%)	GER (%)
50	99.6 A
75	98.7 B
CV (%)	1.23

Means followed by different uppercase letters, in the column, differ from each other by the F test ($p<0.05$)

Silva *et al.* (2018) point out that employing water-retaining gel to maximize water usage in agriculture is an extremely interesting practice, since the storage of water and nutrients and preventing a rapid loss of moisture to the atmosphere or even leaching promotes greater water efficiency in the crop and allows for a more sustainable agriculture.

Considering the minimum germination of 70% for the commercialization of lettuce seeds (Nascimento, 2002), it can be seen that, regardless of treatment, the seeds produced in the present study reached germination values higher than the minimum standard required by Brazilian legislation, ranging from 98.7 to 99.6%. According to Grzybowski *et al.* (2015), seeds with germination between 96 and 100% can be classified as high quality, and according to Conus *et al.* (2009), adaptation to stress can be detected by germination and growth under these conditions.

Comparing the treatments with the control using Dunnett's test at 5% for seed germination (Table 2), it can be seen that, with the exception of the treatment in which 75% of moisture in the substrate was used at a dose of 50 kg ha^{-1} of N, with the application of water-retaining gel, the other treatments did not present any significant differences in relation to the control, which consisted of seeds produced in conditions of 100% of moisture in the substrate associated with the standard dose of nitrogen (100 kg ha^{-1} of N) and without the addition of water-retaining gel, showing that the application of the water-retaining gel possibly led to a greater availability of N for the plants and greater efficiency in water usage.

Table 2. Germination (GER) and germination speed index (GSI) of cultivated lettuce seeds as a function of moisture levels, nitrogen doses and water-retaining gel

Moisture (%)	N doses(kg ha^{-1})	GER (%)	GSI
50	0	98.5 ns	38.6**
	25	100 ^{ns}	44.7**
	50	99.5 ns	45.2**
	100	100 ^{ns}	47.2**
	200	100 ^{ns}	37.7 ns
75	0	99.5 ns	45.2**
	25	98.5 ns	47.3**
	50	97.5*	47.3**
	100	100 ^{ns}	44.5**
	200	98 ^{ns}	27.1 ns
100***	100	100	30.25
CV (%)		1.39	18.72

Means followed by *,** differ statistically from the control ($p<0.05$) and ($p<0.01$), respectively, and means followed by ns do not differ statistically from the control ($p>0.05$) by Dunnett's test.
***Control with 100% moisture, 100 kg ha^{-1} and not using water-retaining gel.

Just as in the present study, Marini *et al.* (2009) also did not find any significant effect as to N doses on lettuce seed germination, obtaining a germination rate of 99%, regardless of treatment. On the other hand, Moraes (2015), studying wheat seed germination as a function of different doses and sources of urea with polymers, observed that higher doses are toxic to the crop, and that the higher the percentage of nitrogen fertilizers, the lower the germination percentage of the seeds.

For the GSI, there was a significant difference among the moisture levels when the dose of 0 kg ha^{-1} of N was used, with a higher index for the moisture level at 75% field capacity (Table 3). At the dose of 200 kg ha^{-1} of N, the highest GSI was found in seeds produced with moisture at 50% field capacity, with 33.7. At the other doses of N, there was no effect on the studied moisture levels, highlighting the efficiency in water usage with the application of the water-retaining gel.

According to Carvalho and Nakagawa (2012), any factor that affects the development and accumulation of reserves by the seed will cause damage to its vigor, highlighting water availability among these factors. However, in the present research work, even the treatments in which the seeds were produced with less water availability obtained better results, for both germination and GSI, a fact that may be associated with the presence of the water-retaining gel in these treatments. This polymer, when incorporated into the soil or substrate, increases water and nutrient retention, reducing eventual leaching of essential nutrients

Table 3. Germination speed index (GSI) of lettuce seeds grown under different levels of moisture and nitrogen doses, using water-retaining gel

Moisture (%)	N doses (kg ha^{-1})				
	0	25	50	100	200
50	38.6 B	44.7 A	45.2 A	47.2 A	33.7 A
75	45.2 A	47.3 A	47.3 A	44.5 A	27.1 B
CV (%)	6.01				

Means followed by different uppercase letters, in the column, differ from each other by the F test ($p<0.05$), as a function of the interaction of the 'moisture levels' factor within the 'nitrogen doses' factor.

and deep percolation of irrigation water (Fernandes *et al.,* 2015). Furthermore, it slowly makes nutrients available to plants, in accordance with the absorption-release cycles (Bernardi *et al.,* 2012).

Studying the effect of different irrigation levels and different doses of water-retaining solution on lettuce production in a protected environment, Silva (2018) observed that the properties of the soil conditioner hydrogel reflect in greater water retention in the soil, reducing losses by percolation and evaporation, thus keeping the soil more moist and allowing plants to develop, using water more efficiently.

Santos and Silva, (2016), while working with the application of commercial water-retaining gel (Terracottem®) in the cultivation of the *Jatropha curcas* plant under different irrigation levels, suggested that applying the gel can reduce water usage by up to 50% without affecting the growth of the plants.

Analyzing the breakdown of N doses within each moisture level,it is possible to observe that the GSI results fit a quadratic behavior regression model for the two moisture conditions (Figure 1). For both moisture levels, there were increases in GSI values followed by decreases, with maximum GSI at the dose of 93.85 kg ha^{-1} of N (48.11), when seeds were produced with 50% moisture. Such results evidence that the seeds germinated faster with a higher degree of uniformity, being therefore considered more vigorous.

At a moisture level of 75%, the dose of 48.25 kg ha^{-1} of N provided the highest germination speed rate (47.41). These results show the greater vigor of the lettuce seeds with the use of N up to adequate levels, and that excessive doses of N provide less vigor.

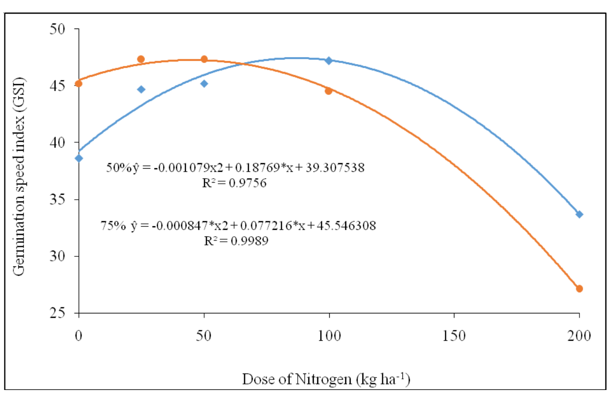

Fig. 1. Germination speed index (GSI) of lettuce seeds as a function of nitrogen doses and substrate moisture levels (50 and 75%).

Analyzing the GSI of the seeds in the studied treatments in comparison with the control, whose seeds were produced in a substrate with 100% moisture, at a dose of 100 Kg ha^{-1}, without the application of water-retaining gel (Table 2), there was a significant difference ($P<0.01$) for most treatments. There is a difference in the seeds produced with the two moisture levels (50 and 75%) and with the doses of N from 0 to 100 Kg ha^{-1}, compared to the control, which obtained higher GSI means, indicating that the seeds from these treatments showed higher germination speeds compared to the control, that is, they germinated faster and uniformly. However, there was no significant difference for the dose of 200 kg ha^{-1} of N for the two moisture levels, denoting that higher doses of N provide lower seed vigor.

In the electrical conductivity evaluation (Table 4), Dunnett's test at 5% shows that, in comparison with the control, there was a significant difference for the seeds produced in substrate with 50% moisture, at the doses of 25 and 200 kg ha^{-1} of N, and for the moisture level at 75% field capacity, at doses of 0 and 25 and 200 kg ha^{-1} of N, using water-retaining gel, when the lowest values for conductivity were found. Lower electrical conductivity values indicate the better vigor of the seeds, due to the lower leaching of their cellular contents. On the other hand, higher conductivity values represent greater leaching of solutes, which is associated with a lower degree of membrane structuring, corresponding to the lower vigor of the seed batch (Marcos Filho, 2015).

Abrantes *et al.* (2010), while working with N doses in top-dressing fertilization and evaluating the quality of millet seeds, concluded that an increase in nitrogen fertilization did not result in structural changes in the membranes and, consequently, did not change the leaching of electrolytes from the seeds, as found in the present study, which did not observe any significant effect for the electrical conductivity variable on the N doses used.

With regard to accelerated aging, the treatments did not differ statistically from the control, with average values ranging from 84.5 to 98.5% of seeds germinated after aging (Table 4), indicating that, by applying water-retaining gel, the substrate moisture and nitrogen dose can be reduced, without affecting the physiological quality of the producedseeds. Possibly, when added to the substrate, the water-retaining gel provided greater efficiency in the use of nitrogen by the plants.

Kolchinski and Schuch (2004) observed this same behavior when comparing nitrogen doses in white oat; however, according to Favaro *et al.* (2011), using higher nitrogen doses allows for greater vigor in seeds subjected to accelerated aging, and this fact may be related to a higher protein content that nitrogen provides, improving the quality of membranes and a greater content of reserves (Imolesi *et al*., 2001).

The accelerated aging test is recognized as one of the most important to evaluate the vigor of seeds of several species, being able to provide information with a high degree of consistency. In this test, it is considered that batches of high-vigor seeds maintain their viability when subjected, for periods of time, to severe conditions of temperature and relative air humidity (Marcos Filho, 2015).

Table 4. Electrical conductivity (EC) and accelerated aging (AA) of cultivated lettuce seeds as a function of moisture levels, nitrogen doses and water-retaining gel

Moisture(%)	N doses (kg ha^{-1})	EC (μS cm^{-1} g^{-1})	AA (%)
50	0	183.3 ns	97 ^{ns}
	25	149.8*	97.5 ns
	50	172.7 ns	93.5 ns
	100	161.1 ns	97.5 ns
	200	149.8*	95 ^{ns}
75	0	148.5*	98 ^{ns}
	25	106.3**	94.5 ns
	50	154.1 ns	94 ^{ns}
	100	170.4 ns	84.5 ns
	200	154.8 ns	99 ^{ns}
100***	100	212.2	98.5
CV (%)		23.03	9.59

Means followed by *,** differ statistically from the control ($p<0.05$) and ($p<0.01$), respectively, and means followed by ns do not differ statistically from the control ($p>0.05$) by Dunnett's test.
***Control with 100% moisture, 100 kg ha^{-1} and not using water-retaining gel

The main purpose of vigor tests, such as accelerated aging, is to verify the physiological quality of seeds when they are subjected to specific environmental conditions or stress (Binotti *et al*., 2008).

In general, the results of the present study indicate that using water-retaining gel in the substrate proved to be a promising alternative for growing lettuce with the purpose of producing seeds, which allows optimizing the use of water associated with reduced doses of nitrogen, without affecting the physiological quality of the produced seeds, because even though the plants were subjected to factors that could affect their development and accumulation of reserves, they obtained satisfactory results.

CONCLUSION

The application of water-retaining gel to lettuce crops helps the plants use the nitrogen fertilization and the moisture available to them more efficiently, with a view to obtaining quality seeds.

The use of water-retaining gel associated with nitrogen fertilization enables the use of lower levels of moisture in the substrate (50%), without affecting the quality of the producedseeds.

N doses from 50 to 100 kg ha^{-1} with the use of water-retaining gel favors the production of seeds with greater vigor.

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