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Application of calcium and boron directed to inflorescences in production, quality and nutrient accumulation in lettuce seeds

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ABSTRACT

Calcium and boron are important nutrients in the stages of flowering, pollen germination and in the fruiting process. As these nutrients are poorly mobile in the plant, some authors recommend foliar spraying in the production of seeds of some species. The objective was to study the influence of calcium and boron, via application directed to inflorescences, in production, quality and accumulation of nutrients in lettuce seeds. Four treatments were evaluated (only calcium: 0.72 g/L; only boron: 0.17 g/L; joint application of calcium and boron and control), in randomized blocks design with six replications. No influence was obtained between the treatments for seed production (number and weight of seeds per plant). Using boron, alone or together with calcium, the weight of a thousand seeds was lower compared to control, while only calcium did not differ from the control. No differences were obtained for germination and seed vigor. The application of calcium did not affect the accumulation of nutrients, however boron application increased the accumulation of this nutrient in the seeds. The application of calcium and boron directly in the inflorescences of lettuce did not promote an increase in the production and vigor of the seeds.

Keywords: *Lactuca sativa*, foliar fertilization, germination, vigor, seed physiological potential.

RESUMO

Produção, qualidade e acúmulo de nutrientes em sementes de alface com aplicação de cálcio e boro direcionada às inflorescências

O cálcio e o boro são nutrientes importantes nos estádios de florescimento, germinação do pólen e no processo de frutificação. Por serem nutrientes pouco móveis na planta, alguns autores recomendam a pulverização foliar na produção de sementes de algumas espécies. Objetivou-se estudar as influências do cálcio e do boro, via aplicação direcionada às inflorescências, na produção, qualidade e acúmulo de nutrientes nas sementes de alface. Foram avaliados quatro tratamentos (apenas cálcio: 0,72 g/L; apenas boro: 0,17 g/L; aplicação conjunta de cálcio e boro e controle), no delineamento em blocos casualizados, com seis repetições. Não houve influência dentre os tratamentos para produção de sementes (número e massa de sementes por planta). Quando se usou boro, sozinho ou em conjunto com o cálcio, a massa de mil sementes foi menor em comparação com o controle, enquanto com apenas cálcio não diferiu do controle. Também não foram obtidas diferenças para germinação e vigor das sementes. A aplicação de cálcio não afetou o acúmulo de nutrientes, no entanto a de boro aumentou o acúmulo desse nutriente nas sementes. A aplicação de cálcio e boro diretamente nas inflorescências da alface não promoveu aumento na produção nem no vigor das sementes.

Palavras-chave: *Lactuca sativa*, adubação foliar, germinação, vigor, potencial fisiológico da semente.

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There is an increase in the crop cycle for the production of lettuce seeds, differing in relation to the production of lettuce for fresh consumption, with the possibility of greater extraction of nutrients by the plant due to the formation of structures such as flowers and fruits (Kano *et al.*, 2011a; Quadros *et al.*, 2012; Martins *et al.*, 2022). Although there are studies about nutrition and fertilizer

recommendations for the commercial cultivation of lettuce and other vegetables, studies that address the effects of nutrients on seed production and quality are much less common (Albornoz *et al.*, 2019; Martins *et al.*, 2022).

A well-nourished plant is able to produce more well-formed seeds (Carvalho & Nakagawa, 2012). At the beginning of the reproductive

phase, the nutritional requirement increases, compared to lettuce for leaves consumption, accumulating up to three times more macronutrients and 50% more micronutrients (Kano *et al.*, 2011a; Sala & Nascimento, 2014).

Calcium (Ca) is a macronutrient associated with structure, cell wall regulation and maintenance of the plasma membrane, being essential for plant growth and development

(Sala & Nascimento, 2014). Boron (B) is an important micronutrient in sugar translocation and carbohydrate metabolism, with functions in the flowering and fruiting process (Malavolta *et al.*, 1997).

Both nutrients, calcium and boron, are important for pollen grain germination and pollen tube growth and are fundamental in flower fertilization and fruit set rate (Leite *et al.*, 2011). As they are preferably distributed via xylem and, due to the low mobility in the plant, Nakada-Freiras *et al.* (2021) suggest that the application of calcium and boron should be done via foliar spraying in the flowering or post-flowering phase in order to have a better use and, consequently, an increase in seed yield.

Foliar fertilization is indicated when it is necessary to make nutrients quickly available, when there is a high demand for the plant depending on the phenological stage, especially with low mobility elements, such as calcium and boron, and in organs with low respiratory rate (Fernández *et al.*, 2015), as flowers and fruits in lettuce.

Epidermal structures that make up the surface of leaves and fruits are permeable to the applied solution. The nutrient must pass through the leaf cuticle, cell wall and plasma membrane in order to reach the cytoplasm. Upon arrival, it can cross the tonoplast to reach the vacuole, or it can move via plasmodesma until it reaches the phloem and then be transported to the places of consumption (Fernández *et al.*, 2015).

Due to the scarce information about the effects of calcium and boron application on seed development and quality in vegetables, the objective was to study the influence of these nutrients via application directed to the inflorescences on the production and quality of lettuce seeds, as well as on the accumulation of nutrients in the seeds.

MATERIAL AND METHODS

The experiment was carried out at the São Manuel Experimental Farm, belonging to the School of Agriculture

(FCA) of the Sao Paulo State University (UNESP), Botucatu campus, located in the municipality of São Manuel-SP (22°25'S, 48°34'W, altitude 750 m).

The experimental area soil is classified as Typical Dystrophic Red Latosol, sandy texture. Chemical analysis (0-20 cm) showed: pH (CaCl₂) = 5.8; organic matter = 15 g/dm³; P = 187 mg/dm³; H+Al = 16 mmol/dm³; K = 3.7 mmol/dm³; Ca = 88 mmol/dm³; Mg = 13 mmol/dm³; B = 0.50 mmol/dm³; sum of bases (SB) = 105 mmol/dm³, CEC = 121 mmol/dm³ and base saturation (V%) = 87%. The calcium content (88 mmol/dm³) is considered high, however because the distribution of calcium in the plant is via xylem, the distribution rate for fruits and seeds is very low, due the low transpiratory rate of these organs, justifying the use in calcium rich soils (Kano *et al.*, 2010), while that of boron is medium (0.50 mg/dm³) (Raij *et al.*, 2001). There was no need to lime the area, as the base saturation was above the ideal (80%) for lettuce production. The planting fertilization, except the boron recommendation, which was not used, and topdressing were carried out according to the recommendations for the State of São Paulo (Trani & Raij, 1996).

NPK (4-14-8, 120 g/m²) and chicken manure (2 kg/m²) were used in planting fertilization. Potassium nitrate was used as nitrogen source for the topdressing fertilization, requiring 57 g/m² divided into five applications. The fertilizer was dissolved and distributed 200 mL per plant, corresponding to the dose of nitrogen required per plant. The first application was carried out 49 days after sowing (DAS) and the others were distributed every 7 days.

Lettuce cultivar Vera (crisp type) was studied. Seeds were sown on September 19, 2018 in 200 cells polypropylene trays, containing the commercial substrate Carolina Soil®. The seedlings were transplanted 26 (DAS), in beds, spaced 1.0 m between rows and 0.5 m between plants. Seedlings were conducted in protected cultivation in an arc-type greenhouse, with 2.5 m ceiling height, 7 m wide x

20 m long, covered with a high-density polyethylene film (150 µm) and sides with anti-aphidic screen. The drip irrigation method was used, with 0.5 m spacing between the drippers.

Four treatments were evaluated, only calcium, only boron, joint application of calcium and boron and control, in a randomized block experimental design, with six replications and five plants per plot, considering useful the three central plants.

The dose, at each application, was 3 g/L of calcium chloride (24% of Ca), which corresponds to 0.72 g/L of Ca²⁺ and for boron it was 1 g/L of boric acid (17% of B), that is, 0.17 g/L of B. The doses of calcium and boron were defined considering the doses used by other authors in other species due to having no recommendation for lettuce seed production. At the beginning of flowering, at 89 DAS, applications began, with spraying only directed to the flowers and seeds in formation, twice a week, using a conventional manual sprayer, with a flow rate of 0.005 L/s. The last application was performed at 117 DAS, totaling nine applications.

Harvest was defined as the moment when the plant had about 80 to 90% of the seeds in physiological maturity, becoming visible white bristles ("papus") (Sala & Nascimento, 2014). Harvest began on January 18, 2019 (121 DAS) and ended on January 31, 2019 (134 DAS). After harvest, the plants were kept in a dry chamber (40% relative humidity and 20°C temperature). After drying (approximately 7% water content), the seeds were separated and cleaned in sieves and by the density seed separator (model 'De Leo Tipo 1'), placed in paper bags and stored in the same dry chamber.

The obtained seeds were evaluated in two periods: right after processing and after nine months of storage in permeable packages, kept in a dry chamber (40% relative humidity and 20°C). Right after processing, the weight of a thousand seeds was evaluated and performed the germination test

(obtaining the germination percentage in the first count and total germination, as well as the germination speed index). After nine months of storage, the same evaluations were carried out, adding the emergence test at 7 and 21 DAS, the emergence speed index, the number of leaves and the fresh matter weight of the seedlings obtained at 21 DAS.

The evaluation of a thousand seeds weight, the germination test (first count and total germination) were carried out as determined by the Seed Analysis Rules (Brasil, 2009). The germination speed index (GSI) was calculated using the daily data collected from the germination test, according to Maguire (1962).

The emergence test was carried out in a greenhouse located in the nursery of the Department of Natural Resources (FCA/UNESP). Seeds were sown in polypropylene 200-cell trays containing Carolina Soil® commercial substrate, placing two seeds per cell, and four replicates of 50 cells per treatment were evaluated. Thinning to one seedling per cell was performed at 7 DAS. Daily emergency assessments were performed up to 21 DAS. The emergence speed index (ESI) was evaluated up to the 7 DAS (Maguire, 1962). The fresh matter weight and number of leaves of 30 seedlings per plot were evaluated at 21 DAS. The samples were weighed on an analytical scale with a precision of 0.001 g, and the fresh weight results were expressed in g/plant.

To estimate the accumulation of nutrients in seeds per plant, first the

nutrient contents in seed samples of the six replications (blocks) were determined for each treatment. The samples were sent to the FCA/UNESP plant chemical analysis laboratory to determine the macro and micronutrient content in the seeds. The chemical analysis methodology was sulfur digestion for nitrogen determination, dry digestion for boron and nitric-perchloric digestion for other macro and micronutrients (Malavolta *et al.*, 1997).

After the determination of nutrients content, the accumulation of nutrients was obtained by the proportionality of the nutrient content with the dry weight of seeds produced per plant, expressing the values in mg/plant.

Data were subjected to analysis of variance and means compared by Tukey test at 5% probability. Data were processed by the SISVAR 5.7 software, Program for Statistical Analysis and Planning of Experiments at the Federal University of Lavras (Ferreira, 2019).

RESULTS AND DISCUSSION

There was no difference between treatments for seed production, both in weight and number of seeds per plant (Table 1). It can be observed that the values obtained for weight (20.3 g/plant) and number (average of 21,932) of seeds per plant are close to, or even above, those found in the literature (Kano *et al.*, 2012; Quadros *et al.*, 2012; Sala & Nascimento, 2014; Claudio *et al.*, 2020; Martins *et al.*, 2022) and can be considered high, probably due to the protected cultivation that reduces the incidence of pests and diseases, in addition to avoiding wind and rain during the seed maturation phase that could cause the dispersion of seeds.

Even the control presented high seed production, showing that there were no restrictions to the good development of the plants. The calcium content (88 mmol/dm³) in the soil was high and boron content (0.50 mg/dm³) was medium, according to Rajj *et al.* (2001). Although inorganic

Table 1. Average production (weight and number) of seeds per lettuce plant, as a function of the application of calcium and boron directed to flowers and seeds. São Manuel, FCA/UNESP, 2019.

Treatments	Seed weight (g/plant)	Seed number (units/plant)
Control	19.13 a	20,245 a
Calcium (Ca)	17.98 a	19,791 a
Boron (B)	22.46 a	25,016 a
Ca + B	21.73 a	22,675 a
CV(%)	15.88	16.26

Averages followed by same letters in the column do not differ by Tukey test, 5% probability.

Table 2. Average weight of a thousand seeds (WTS), first count (FC), total germination (TG) and germination speed index (GSI), obtained right after processing and after nine months of seed storage, as a function of the application of calcium and boron directed to flowers and seeds. São Manuel, FCA/UNESP, 2019.

Treatments	Right after processing				9 months after storage			
	WTS (g)	FC (%)	TG (%)	GSI	WTS (g)	FC (%)	TG (%)	GSI
Control	0.96 a	73 a	94 a	11.13 a	0.95 a	83 a	96 a	12.34 a
Calcium (Ca)	0.94 ab	81 a	96 a	11.64 a	0.96 a	78 a	97 a	13.02 a
Boron (B)	0.91 b	77 a	92 a	11.10 a	0.90 b	89 a	96 a	13.02 a
Ca + B	0.90 b	85 a	95 a	11.54 a	0.89 b	78 a	98 a	12.99 a
CV(%)	2.84	19.08	5.09	7.04	3.33	15.98	3.10	6.78

Averages followed by same letters in the columns do not differ by Tukey test at 5% probability.

fertilizers containing these nutrients were not used in the base fertilization, chicken manure (20 t/ha) was used, according to the recommendation of Trani & Raji (1996), which is a source of these nutrients, in addition to irrigation carried out throughout the cycle, providing ideal conditions for absorption of these nutrients.

Nakada-Freitas *et al.* (2020) also did not observe the effect of boron application at different stages of the cauliflower plant (*Brassica oleracea* var. botrytis) on seed production. However, Nakada-Freitas *et al.* (2021) reported an increase in cauliflower seed production with foliar application of calcium (the same concentration as in this research) at the seed maturation stage: after beginning of flowering. Javorski *et al.* (2015) and Silva *et al.* (2017) observed, in corn (*Zea mays*) and physalis (*Physalis peruviana*), respectively, an increase in the number of seeds with application of boron and calcium, justifying these results by the optimization of pollen grain germination. Boron is used in foliar application to increase production and fruit quality of some vegetables (Javorski *et al.*, 2015; Nakada-Freitas *et al.*, 2021) and is of great importance in seed production, as it acts in the process of pollen grain formation and tube pollen growth (Leite *et al.*, 2011). However, it did not affect lettuce seed production at the concentration and stage in which it was applied.

The translocation of calcium and boron occurs along with water in the xylem, being directly influenced by the transpiration rate of the plant. As flowers, fruits and seeds have a small transpiration surface and a high growth rate, they are more susceptible to deficiency of these nutrients. In this way, the supply of calcium and boron can be inefficient via soil or nutrient solution; so, application directed to these organs is a good option for supplementation, which can correct the lack of these nutrients with low mobility in the phloem (Marschner, 2012; Fernández *et al.*, 2015).

The extent mobility of B depends on the species; these are classified as those

with restricted B mobility and those in which B is highly mobile. Mobility occurs in some species that produce simple sugars as polyols (sorbitol, manitol, or dulcitol). These sugars can effectively complex B, resulting in polyol-B-polyol products, which may be transported by the phloem to the growing points such as vegetative and reproductive meristems (Hu *et al.*, 1997). The polyols are present in many species, but we do not found mention in lettuce.

The treatments in which boron was used, alone or together with calcium, resulted in a smaller weight of one thousand seeds compared to the control in both evaluations: after processing and after nine months of storage (Table 2). Boron acts in the translocation of sugars to propagative organs (Marschner, 2012) which should provide an increase in seed weight. However, the opposite was observed, probably because this nutrient has the narrowest limit between deficiency and toxicity (Malavolta *et al.*, 1997), and there may have been an adverse effect on the accumulation of dry weight in seeds due to excess boron (Nakada-Freitas *et al.*, 2020). Eggert & Wirén (2016) found that oilseed rape seeds with higher internal boron content generated plants with lower fresh matter, indicating an inhibitory effect of boron on growth.

No differences were observed between treatments for quantitative and qualitative characteristics related to seed quality (Tables 2 and 3). Therefore, it is observed that the application of calcium and boron did not affect the physiological quality of the seeds.

Some authors report a correlation between the weight of a thousand seeds and the physiological quality of the lot, due to the amount of energy reserve (Carvalho & Nakagawa, 2012), which was not observed in this research. Despite the differences for the weight of a thousand seeds, the values observed, ranging from 0.89 to 0.96 g (Table 2), are within the range reported by other authors (Cardoso *et al.*, 2011; Kano *et al.*, 2012; Quadros *et al.*, 2012; Sala

& Nascimento, 2014; Claudio *et al.*, 2020), who mention averages of 0.90 g to 1.15 g, that is, there was no drastic reduction in seed weight. Cardoso *et al.* (2016) reported a linear increase in the weight of a thousand cauliflower seeds with the increase in phosphorus doses without, however, affecting their physiological quality, that is, it is not uncommon in fertilization research to observe effect for the weight of a thousand seeds without affecting the germination and vigor.

Several authors have reported that the physiological quality of lettuce seeds is not reduced even with severe nutritional deficiencies (Cardoso *et al.*, 2011; Kano *et al.*, 2012; Quadros *et al.*, 2012), especially when evaluating benefited seeds, as the empty and poorly formed are eliminated during the process of impurities remotion with sieves and by difference in density, obtaining seeds that would be commercialized, which justifies good germination and vigor. Kano *et al.* (2011b) observed that the effect of nutritional deficiency on lettuce seed production was only observed after 25 months of seed storage.

The lowest numerical value for germination at 7 DAS, considering the two evaluations (right after processing and nine months of storage) was 92% (Table 2), a value much higher than the minimum required by Brazilian legislation, which is 80% germination (Brasil, 2019). Also, high values were obtained (minimum of 89% at 7 DAS) in the emergence test (Table 3), which demonstrates the excellent quality of the seeds, regardless of the treatments.

All seedlings originated in the emergence test can be considered normal, with all essential structures, that is, with the formation of quality seedlings. So, even reducing the thousand seeds weight, the application of boron did not reduce seed vigor. The seedling quality is a very important factor in the vegetable production chain, since the vigor of the seedling produced directly influences the development and final performance of the plant. Vigorous seedlings usually take less time to reestablish themselves

in the field after transplanting and result in greater production and precocity (Rodrigues *et al.*, 2010).

There was no difference between treatments for the accumulation of all macronutrients in lettuce seeds (Table 4). It was expected that there would be an increase in calcium accumulation in treatments in which this nutrient was applied, but there was no difference in the accumulation of calcium in lettuce seeds, even in treatments with application of this nutrient (Table 4), showing that it is not absorbed by the seed when sprayed direct to the flowers and seeds in formation. According to Kano *et al.* (2011b), calcium is a low mobility element, and accumulation in the seed occurs through absorption and transport during seed maturation. About 35% of calcium in lettuce seeds is absorbed after flowering begins, and it is transported preferentially by the xylem, with no redistribution from leaves to the seeds (Kano *et al.*, 2011a) and, apparently, the calcium was not absorbed by the seeds. In other researches where calcium application was studied, the authors usually did not assess the absorption of the nutrients. In okra (*Abelmoschus esculentus*), Bardivieso (2022) observed greater accumulation of calcium in the fruits when calcium was applied directed to them, but not in the seeds.

Bewley *et al.* (2013) state that the transport of nutrients already assimilated by plants is carried out via phloem to the seed coat or placental region, being released into the extracellular space, which will

Table 3. Average seedling emergence at 7 (E7) and 21 (E21) days after sowing (DAS), emergence speed index (ESI), number of leaves (NL) and fresh matter weight (FMW) per seedling evaluated at 21 DAS, as a function of the application of calcium and boron directed to flowers and seeds. São Manuel, FCA/UNESP, 2019.

Treatments	E7 (%)	E21 (%)	ESI	NL (leaves/plant)	FMW (g/plant)
Control	91 a	99 a	22.97 a	3.25 a	10.6150 a
Calcium (Ca)	91 a	98 a	24.39 a	3.00 a	12.2675 a
Boron (B)	91 a	97 a	23.61 a	3.00 a	9.9225 a
Ca + B	89 a	97 a	22.77 a	3.25 a	10.1125 a
CV(%)	3.74	2.48	4.47	11.31	19.24

Averages followed by same letters in the columns do not differ by Tukey test at 5% probability.

later be imported by the endosperm and seed embryo. The movement of calcium from leaves to tissues fed via phloem, such as fruits and seeds, is low. Probably for this reason, in most vegetables, calcium is among the nutrients with the lowest accumulation in the seeds (Kano *et al.*, 2010; Cardoso *et al.*, 2016; Aguilar *et al.*, 2020, 2021; Tavares *et al.*, 2021; Bardivieso, 2022). The mean descending order of accumulated macronutrients was (mg/plant): N (939.79) > K (237.25) > P (208.92) > Ca (82.92) > Mg (80.23) > S (44.24). Quadros *et al.* (2010) obtained a similar order (N>P>K>Ca>Mg>S), only with the inversion of the orders of P and K.

In all the mentioned researches with lettuce seeds, nutrients N, K and P appear as the most accumulated. According to Cardoso *et al.* (2016), the accumulation of nitrogen, when compared to other macronutrients, tends to be greater due to its importance in the formation of

seeds (mainly proteins), in addition to having easy mobility in the plant, as well as potassium, the second nutrient with the highest accumulation in the seed. Phosphorus is accumulated in the seed in the form of phytin, being important in germination after undergoing the action of the phytase enzyme (Carvalho & Nakagawa, 2012).

For micronutrients, the descending order of accumulation depended on the treatments. For those who received only calcium and the control, the order was: Fe>Zn>Mn>B>Cu, and for treatments containing boron it was Fe>Zn>B>Mn>Cu. According to Kano *et al.* (2011a), although the order is like the cultivation for the market, when it comes to the production of lettuce seeds, the micronutrient requirement is much higher (about 50%) compared to the production of vegetables for the sale of leaves.

If there was no difference for

Table 4. Nutrient accumulation in lettuce seeds produced per plant as a function of the application of calcium and boron directed to flowers and seeds. São Manuel, FCA/UNESP, 2019.

Treatments	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	(mg/plant)										
Control	974a	216.67a	264.56a	86.89a	84.76a	46.54a	0.40b	0.34a	2.56a	0.49a	2.57a
Calcium (Ca)	885a	197.73a	226.49a	83.05a	76.06a	37.52a	0.29b	0.24b	2.41a	0.49a	2.29a
Boron (B)	859a	190.06a	206.47a	71.92a	70.84a	43.54a	0.88a	0.26ab	2.76a	0.39a	2.18a
Ca + B	1040a	231.22a	251.48a	89.81a	89.27a	49.38a	1.15a	0.29ab	3.00a	0.48a	2.61a
CV(%)	18.44	17.75	22.22	18.45	19.99	18.51	32.33	19.74	19.09	28.96	19.10

Averages followed by same letters in the columns do not differ by Tukey test at 5% probability.

calcium, the greatest accumulation of boron in the seeds occurred in treatments where boron was applied to the inflorescences, alone or accompanied by calcium (Table 4), proving that this nutrient was absorbed by the seeds, unlike calcium, and consequently promoted greater accumulation. In lettuce seed production, boron is absorbed after the beginning of flowering and during the period of seed maturation (Kano *et al.*, 2011a), which was the period of application of treatments. Greater accumulation of boron was related in okra seeds (Rahman *et al.*, 2017), and oilseed rape (*Brassica napus*) (Eggert & Wirén, 2016), and, according to Landi *et al.* (2019), it occurs because boric acid is an uncharged molecule, and it can pass through lipid bilayers without any degree of control, leading to excess in the plant.

It can be concluded that the application of calcium and boron directly to the lettuce inflorescences did not affect the production and vigor of the seeds. However, the application of boron reduced the thousand seed weight and increased the accumulation of boron in the seeds, while the application of calcium did not affect the accumulation of any nutrient in the seeds.

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