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## Calcium and boron foliar application in the production and quality of sweet pepper seeds

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### ABSTRACT

Calcium and boron are important nutrients in pollen grain formation and seed production. As they are little mobile in plants, it is common to be supplied via foliar application. The objective of this research was to evaluate the production and quality of sweet pepper seeds with calcium and boron application directed to flowers and fruits. The experimental design was in randomized blocks, with seven replications. Four treatments were evaluated: isolated calcium (0.2%) application, isolated boron (0.1%) application, combined application of calcium (0.2%) and boron (0.1%), and the control without calcium and without boron. The characteristics evaluated were number of pollen grains, number of seeds per fruit; 1000 seed weight; physiological quality of seeds (germination, first germination count, and germination speed index) and protein (albumin, globulin, prolamine and glutelin) contents in seeds. Both the application of calcium and boron did not affect the physiological quality of the seeds. Boron application reduced seed production and the 1000 seed weight, while calcium application increased the number of pollen grains, seed production and seed albumin content, and, therefore, the application of calcium in the production of sweet pepper seeds is recommended.

**Keywords:** *Capsicum annuum*, germination, vigor, pollen, protein.

### RESUMO

#### Aplicação foliar de cálcio e boro na produção e qualidade de sementes de pimentão

O cálcio e o boro são nutrientes importantes na formação do grão de pólen e na produção de sementes. Por serem pouco móveis nas plantas, é comum o fornecimento via aplicação foliar. Objetivou-se com este trabalho avaliar a produção e qualidade das sementes de pimentão em função da aplicação de cálcio e boro direcionado às flores e frutos. O delineamento experimental foi em blocos casualizados, com sete repetições. Foram avaliados quatro tratamentos: aplicação isolada de cálcio (0,2%), aplicação isolada de boro (0,1%), aplicação conjunta de cálcio (0,2%) e boro (0,1%) e o controle sem cálcio nem boro. Foram avaliadas as características número de grãos de pólen, número de sementes por fruto; massa de 1000 sementes; qualidade fisiológica das sementes (germinação, primeira contagem de germinação e índice de velocidade de germinação) e teores de proteínas (albumina, globulina, prolamina e glutelina) nas sementes. Tanto a aplicação de cálcio como de boro não afetaram a qualidade fisiológica das sementes. A aplicação de boro reduziu a produção de sementes e a massa de mil sementes, enquanto a de cálcio aumentou a produção de grãos de pólen e de sementes e o teor de albumina nas sementes e, portanto, recomenda-se a aplicação de cálcio na produção de sementes de pimentão.

**Palavras-chave:** *Capsicum annuum*, germinação, vigor, pólen, proteínas.

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Seeds of good quality ensure better germination, rapid and uniform emergence and vigorous seedling growth, under a wide range of environmental conditions during emergence, which may affect the productivity and quality of the commercial product (Carvalho & Nakagawa, 2012). In vegetables, producers have demanded higher quality seeds than official standards, due to the high value of seeds, especially hybrids.

The physiological quality of seeds of

the genus *Capsicum* depends of several factors, especially fruit maturation and post-harvest rest (Colombari *et al.*, 2021). Another factor that can affect seed quality is plant nutrition, as well-nourished plants are capable of producing more seeds with superior quality, and the seed maturation period is the one with the greatest demand for nutrients by the plants (Carvalho & Nakagawa, 2012; Cardoso *et al.*, 2016a). Despite the importance of plant nutrition

in seed production, there are few studies about the effects of nutrients on vegetable seed production and quality (Cardoso *et al.*, 2016b).

Fruits and seeds are the main drains in a plant, being the nutrients carried to their formation and, if deficient in the soil, many nutrients can be translocated from the leaves to the seeds (Cardoso *et al.*, 2016a; Freitas *et al.*, 2021). However, due to the distribution of calcium (Ca) and boron (B) in the

plant being preferably via xylem, the rate of redistribution for the fruits and seeds is very low (Oliveira *et al.*, 2015). For this reason, Fernández *et al.* (2013) suggested that the supply of these nutrients should be done via foliar application in the flowering or post-flowering phase in order to increase seed yield and quality. Besides this, calcium and boron are nutrients of great importance in germination of pollen, pollen tube growth, flower fixation and, therefore, in seed production (Salim *et al.*, 2019), and foliar application permits to control the quantity of the nutrients and the specific period of plant growth (Niu *et al.*, 2020).

In peppers, calcium is the third most accumulated nutrient by plants (Lima *et al.*, 2018), and its deficiency can lead to fruit apical rot. Boron is the micronutrient most accumulated by pepper fruits and, in adequate amounts, it can favor the development and production of fruits and its deficiency can lead to floral abortion and poor seed formation (Mello *et al.*, 2002). Zeist *et al.* (2018) and Salim *et al.* (2019) related greater fruit yield in pepper plants with foliar application of calcium and boron, isolated or combined, but they did not evaluate seed production.

There are several studies in which the application of calcium and boron are studied in many species, but information on vegetables, mainly with the goal of seed production, is scarce. Considering the high commercial value of sweet pepper seeds, the need to obtain high quality seeds and the importance of nutrition in seed production, the objective of this research was to evaluate the effect of calcium and boron application directed to flowers and fruits on the production and quality of sweet pepper seeds.

## MATERIAL AND METHODS

The experiment was carried out in the São Manuel Experimental Farm (22°46'S, 48°34'W and 740 m altitude), located in the municipality of São Manuel, Brazil, belonging to the School of Agriculture of the São Paulo State University. The plants were cultivated in an arc-type greenhouse with 7x20 m and

3 m height, covered with a low-density polyethylene film (150 µm thickness), and the structure had its laterals totally closed with an anti-aphid screen. In the period of the experiment the maximum daily temperature was 29.5°C and the minimum was 18.3°C and the maximum and minimum relative humidity were 82 and 49%, respectively, inside the protected environment.

Plants were conducted in 12-liter pots with corrected and fertilized soil as recommended by Boletim Técnico 100 (Raij *et al.*, 1997). The soil (typic Distrophic Red Latosol, sandy texture) chemical analysis showed the following results (before fertilization):  $\text{pH}_{(\text{CaCl}_2)}$  = 4.5; organic matter = 4 g/dm<sup>3</sup>; P = 4 mg/dm<sup>3</sup>; H+Al = 9 mmol<sub>c</sub>/dm<sup>3</sup>; K = 0.4 mmol<sub>c</sub>/dm<sup>3</sup>; Ca = 13 mmol<sub>c</sub>/dm<sup>3</sup>; Mg = 3 mmol<sub>c</sub>/dm<sup>3</sup>; sum of bases (SB) = 17 mmol<sub>c</sub>/dm<sup>3</sup>; CEC = 26 mmol<sub>c</sub>/dm<sup>3</sup> and base saturation (V%) = 64%.

Four treatments were evaluated: isolated calcium application, isolated boron application, combined application of calcium and boron, and the control without calcium and without boron. Calcium was applied at a dose of 0.2% and boron at 0.1%. Doses were defined based on the recommendations by Melo *et al.* (2014) for commercial production of tomato, which belongs to the same family. As source of calcium and boron, calcium chloride (27% of Ca) and boric acid (17% of B) were used, respectively. Applications were directed to flowers and fruits, once a week, between 8:00 to 9:00 h am, starting when the first flowers were about to reach anthesis and ending when the fruit color change (green to red) started. The experimental design was in randomized blocks, with seven replications and five plants per plot, considering three central plants as useful plot.

Sowing of line SK 1730 (Sakata Seed Sudamerica®) was performed in 162-cell polypropylene trays containing Carolina Soil® substrate. Seedlings were transplanted with four leaves. The sprouts were removed until the appearance of the first flower, and topdressing by fertigation according to Trani *et al.* (2011) recommendations.

Fruits were harvested when 50%

of the surface was red, and, after, they were left to rest for seven days, according to Colombari *et al.* (2021). Seeds were extracted manually, cutting the fruits longitudinally and, after extraction, the seeds were stored in a dry chamber (40% RH and 20°C) in order to standardize the water content at 8%. Afterwards, they were benefited with the aid of a seed separator by density (model 'De Leo Type 1'), being used only the classified seeds to carry out the following evaluations: a) number of seeds per fruit (NSF); b) weight of 1000 seeds (W1000S) [eight repetitions of 100 seeds were sampled and the weight was evaluated, estimating the value for 1000 seeds, according to Brasil (2009)]; c) germination and first count in germination test (FC) [four replicates of 50 seeds were sampled and placed inside a gerbox with two germination papers, according to the Seed Analysis Rules methodology (BRASIL, 2009), and the number of normal seedlings was counted on the 7<sup>th</sup> (first count) and on the 14<sup>th</sup> (germination) day after sowing (DAS), expressed in percentage of germination]; d) germination speed index (GSI) [daily observations were made after installing the germination test, counting the number of seeds germinated per day and calculating the GSI, according to Maguire (1962)]; e) protein (albumin, globulin, prolamine and glutelin) [contents in seeds were determined according to Bradford (1976)].

The number of pollen grains (PG) in each anther was also evaluated, according to methodology described by Nogueira *et al.* (2015). In each plot, five flower buds were collected in the "balloon" stage (one day before anthesis), and the anthers were separated and the pollen grains were counted in an optical microscope (100X objective).

Data were submitted to analysis of variance and Tukey test ( $p < 0.05$ ) was used to compare averages, using the statistical software Sisvar.

## RESULTS AND DISCUSSION

The isolated application of calcium increased the number of seeds per fruit in 17% compared to the control

(Table 1), but it did not differ from the treatment with combined application of calcium and boron. Calcium has several functions in the plant, including development and germination of pollen grains, in addition to pollen tube growth (Silva *et al.*, 2017), which may explain the greater number of seeds obtained per fruit. Probably, this increasing in seed number is due to the increasing in the number of pollen grains with the application of calcium compared to the control in 21%.

The plant's natural action is to distribute photoassimilates and mobile nutrients from vegetative organs (mainly leaves) to the fruits and seeds through the phloem. However, calcium is considered to be a poorly mobile nutrient in the plant and its application directed to flowers and fruits is an alternative to increase seed production (Freitas *et al.*, 2021), as observed in the present research with sweet pepper. Young reproductive structures have low vascular differentiation, which limits calcium flow to flowers and fruits during fruit development and, in sweet pepper, although calcium is the third most accumulated nutrient in the plant, the largest proportion of it is found in the leaves and only a part in the fruits (Oliveira *et al.*, 2015).

The values observed for the number of seeds per fruit (98 to 123 seeds, Table 1) are lower than the values reported by Freitas *et al.* (2015) in sweet pepper cultivar Doce Comprida (minimum of 126 seeds per fruit). However, as reported by the authors, the number of seeds per fruit is a genetic characteristic, which may vary according to the genotype evaluated. In addition, despite the species being

considered autogamous, there may be a reduction in the number of seeds per fruit when cultivated in a protected environment with screens that prevent the entry of pollinating insects, as noted by Freitas *et al.* (2015), obtaining 126 and 259 seeds per fruit when cultivated using screens closing the sides of the greenhouse and without screens, respectively.

On the other hand, the isolated application of boron reduced the production of seeds per fruit (Table 1), showing that this application can be harmful to sweet pepper seed production. Domingos *et al.* (2021) related a quadratic effect of boron doses in soybean seed production, with reduction in yield in the highest doses. High doses of boron can cause toxicity, with reduction in the photosynthetic rates of the plant (Pawlowski *et al.*, 2019) and climatic conditions at the time of application and plant stage can cause different responses (Domingos *et al.*, 2021).

Boron is very important in the production of seeds, as it acts in the process of formation of the pollen grain and in the growth of the pollen tube (Nogueira *et al.*, 2015), what was not noticed in this research as the number of pollen grains in this treatment did not differ from the control. It is the micronutrient that has the narrowest limit between deficiency and toxicity, and, therefore, any error in the used dose can seriously compromise production, which may explain the deleterious effect on the formation of seeds in the present research. There are no recommendations for doses and frequency of foliar application of this micronutrient for sweet pepper seed

production and, probably, the dose and/or frequency used were excessive, causing the deleterious effect in seed production. Zeist *et al.* (2018) related greater fruit production with biweekly application of boron (0.01%) on the leaves from the beginning of flowering, while Salim *et al.* (2019) obtained more fruits with 0.2 to 0.4% of boron. So, the dose evaluated in the present study is in the average of the doses of these cited authors, but the application was more frequent, once a week.

There is no consensus in the literature regarding the effect of application of calcium and boron in seed or fruit production. In tomato, Zamban *et al.* (2018) obtained greater fruit production with application of calcium, but they did not evaluate seed production. Silva *et al.* (2017) observed that both the application of calcium and boron increased the pollen grain germination rate and, consequently, the seed production per fruit in *Physalis*, as well as Nogueira *et al.* (2015) in loquat plants. In cauliflower, Freitas *et al.* (2021) reported an increase in seed production with the application of calcium in the reproductive stage. The contrasting results reported by different authors occur because the researches were carried out with different species (or cultivars), with different doses of the nutrients, and different plant development stages, and more researches are needed to get recommendations for each species. Also, the climatic conditions can influence the absorption process (Fernández *et al.*, 2013).

The application of boron reduced the 1000 seeds weight, compared to the control and to the calcium application (Table 1), confirming the possible

**Table 1.** Number of pollen grains (PG), number of seeds per fruit (NSF), weight of one thousand seeds (W1000S), germination, first count (FC) in the germination test and germination speed index (GSI) of sweet pepper seeds due to the application of calcium and boron directed to flowers and fruits. São Manuel, UNESP, 2019.

Treatments	PG	NSF	W1000S (g)	Germination (%)	FC (%)	GSI
Control	92.3 b	104.6 b	7.8 ab	99.5 a	76.0 a	12.1 a
Calcium	111.3 a	123.3 a	7.9 a	99.3 a	81.0 a	12.1 a
Boron	98.4 b	97.6 c	6.6 c	99.8 a	82.0 a	12.5 a
Calcium + boron	102.6 ab	118.0 ab	7.0 bc	99.8 a	87.0 a	12.6 a

Means followed by same letters, in the columns, do not differ from each other by Tukey test ( $p < 0.05$ ).

deleterious effect of the application of this micronutrient in the dose and/or frequency studied. Also, Domingos *et al.* (2021) related a negative effect of boron foliar application in the 1000 seeds weight of soybean. The values observed for the 1000 seeds weight (6.61 to 7.86 g) are similar to those reported by Colombari *et al.* (2021), which ranged from 6.6 to 7.7 g, that is, these values are normal for the species.

Usually, larger and/or heavier seeds are more vigorous (Carvalho & Nakagawa, 2012). However, this did not occur in the present study, as no differences were observed for germination (mean 99.6%), first count (mean 81.5%) and GSI (mean 12.3) (Table 1), correlation that was also not observed in other vegetables seeds (Cardoso *et al.*, 2016b).

Generally, seeds produced under marginal conditions are usually as viable and vigorous as those produced under more favorable conditions. In this case, the influence of fertilization would be basically on the number of seeds produced, not affecting the quality. The typical response of plants to a nutrient deficiency or excess is a reduction in the amount of seeds produced and, only in more severe conditions occur a reduction in quality. This result, increase or reduction in production without affecting quality, was observed in the present research and by several authors who evaluated fertilization in the production of vegetable seeds (Cardoso *et al.*, 2016b).

According to Cardoso *et al.* (2016b), besides the plausible answer of the plants to adverse conditions aiming the species' perpetuation with vigorous seed production, another point that may be providing no difference in seed quality in researches with nutrients application, is the classification of the seeds. Mostly, the seeds harvested in the experiments are processed, with the removal of the impurities and damaged ones, before the evaluation of the quality. Thus, there is a standardization of seed batches of different treatments regarding physiological quality. It should be noted that this processing is always carried out in order to commercialize the seeds.

**Table 2.** Albumin, globulin, glutelin and prolamin contents in sweet pepper seeds as a function of calcium and boron application directed to flowers and fruits. São Manuel, FCA/UNESP, 2019.

Treatments	Albumin	Globulin	Glutelin	Prolamin
	(mg/g dry matter)			
Control	65.0 b	15.9 a	7.6 a	6.0 a
Calcium	78.3 a	14.4 a	6.2 a	6.3 a
Boron	63.6 b	13.6 a	6.9 a	5.6 a
Calcium + boron	77.3 a	9.1 b	6.9 a	5.9 a

Means followed by same letters, in the columns, do not differ from each other by Tukey test ( $p < 0.05$ ).

For protein content in seeds, no difference was observed for glutelin and prolamin, with averages of 6.91 and 5.94 mg/g of dry matter, respectively (Table 2). On the other hand, the application of calcium and calcium plus boron increased the albumin content, compared to the control and the isolated application of boron, while the application of calcium plus boron reduced the globulin content.

The lower globulin content in pepper seeds, in the treatment with calcium plus boron application, may be related to boron toxicity. Although boron acts in the synthesis of nucleic acids and proteins, excess of this nutrient causes an increase in ribonuclease activity, disturbing the mechanism of synthesis of this protein (Archana & Pandey, 2014; Lee *et al.*, 2015).

Seeds accumulate reserves during their development, mainly carbohydrates and proteins (Carvalho & Nakagawa, 2012), and proteins are classified as albumins, globulins, prolamins and glutelin (Silva *et al.*, 2012). In this research, the decreasing order of protein content in sweet pepper seeds was albumin > globulin > glutelin > prolamin (Table 2).

According to Silva *et al.* (2012), albumins are the main storage protein in seeds of dicotyledonous species, followed by globulins, which was confirmed in sweet pepper seeds, while prolamins are more common in grasses.

Although the effect of nutrients in deposition and mobilization of protein reserves in seeds is not fully understood, it is believed that  $Ca^{+2}$  and

$Mg^{+2}$  cations contribute to these events to happen (Santos *et al.*, 2012), which was observed for albumins, which was the protein found in greatest amount in sweet pepper seeds (Table 2).

Archana *et al.* (2021) studied the effects of boron doses on the expression of proteins (albumin, globulin, glutelin and prolamin) and on the quality of seeds of several species, reporting that at higher doses there were signs of boron toxicity, which caused low protein expression, resulting in low seed quality. Reis *et al.* (2020), in rice seeds, also found a relationship between protein content and seed physiological quality. These authors observed that the reduction in protein content (albumin, globulin, glutelin and prolamin), especially albumin, negatively affected seed production and quality. Similar results were found in this research, since the treatment with isolated application of boron had lower albumin content and also lower production and weight of a thousand seeds (Tables 1 and 2).

According to Fernández *et al.* (2013), a series of variables can interfere with the efficiency of foliar fertilization, such as climatic conditions, nutritional and hydraulic status of the plant, and can explain the discrepancy between different authors, and more researches are necessary to get the ideal technique to calcium and boron foliar application. However, the application of calcium in sweet pepper can be recommended because it increased the number of pollen grains and also the number of seeds per fruit, without affecting the quality of the seeds.

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