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Calcium and Boron Foliar Application in the Production and Quality of Zucchini Seeds

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HIGHLIGHTS

- The application of Ca or B does not affect the germination of zucchini seeds.
- The application of Ca in zucchini increases the number of pollen grains per flower.
- The production of zucchini seeds is favored by the application of Ca.

Abstract: Although there are fertilization recommendations for the cultivation of zucchini, there are just few researches about the effects of nutrients in seed production. Calcium and boron have a low mobility in plants, and some researches recommend foliar application for these nutrients. Thus, the objective of this research was to evaluate the foliar application of calcium and boron in the production and quality of zucchini seeds. The experimental design was randomized blocks with seven replications. Four treatments were evaluated: only calcium (0.2%) application, only boron (0.1%) application, combined application of calcium (0.2%) and boron (0.1%) and the control (without calcium and boron.) The following characteristics were evaluated: number of pollen grains per flower, hundred seeds weight; seed number and weight per fruit and seed physiological quality (germination; first germination count and speed of germination index - SGI). Only boron application reduced the number of seeds produced per fruit. The combined foliar application of calcium and boron, in addition to reducing the number of seeds, also reduced SGI. The calcium only application increased the amount of pollen per flower (superior to all other treatments) and also the number of seeds per fruit (compared to the combined application of calcium + boron), without affecting the quality of the zucchini seeds. So, it can be recomended the aplication of Ca during zucchini flowering for seed production.

Keywords: *Cucurbita pepo* L.; germination; pollen; seed vigor.

INTRODUCTION

Seed quality guarantees better germination, rapid and uniform emergence and vigorous seedling growth, which can even increase the yield and quality of the commercial product [1,2]. Thus, vegetable producers demand high quality seeds, even more in hybrid seeds that have high value.

Plant nutrition is one factor that can affects seed quality because, during their development, the nutrients are translocate to the fruits and seeds, being used as synthesis and reserve material. In this context, during the reproductive phase, plants have a higher nutritional requirement, being critical during seed formation, when stored compounds will have an influence on formation of the embryo and, consequently, in seed vigor [3,4].

Although there are studies and fertilization recommendations for commercial cultivation of various vegetables, there are few studies about the effects of nutrients on seed production and quality [5].

Calcium (Ca) and boron (B) are nutrients of great importance on pollen germination, pollen tube growth, flower fixation and seed production [6–8]. However, due to the distribution of Ca and B in the plant being preferably via xylem, the rate of redistribution for the fruits and seeds is very low [9–11].

Despite the importance of these nutrients, there was not found researches about boron and calcium application in zucchini (*Cucurbita pepo* L.) seed production, an economic expressiveness Brazilian vegetable. Thus, the objective of this research was to evaluate the effect of the foliar application of calcium and boron on the production and quality (germination and vigor) of zucchini seeds.

MATERIAL AND METHODS

The experiment was conducted in a protected environment, at Sítio Modelo, belonging to the School of Agriculture (FCA) of Sao Paulo State University (UNESP), Botucatu Campus - SP. The greenhouse structure is of the arch type with 7.0 x 40.0 m, covered by transparent, low density polyethylene (LDPE) film, 150µm thick and anti-aphidic screen on the sides.

The plants were grown in 13-liter pots. The soil used showed the following chemical characteristics: $pH_{(CaCl2)}$= 4.2; organic matter= 9 g dm^{-3}; P= 2 mg dm^{-3}; H+Al= 31 $mmol_c$ dm^{-3}; K= 0,6 $mmol_c$ dm^{-3}; Ca= 3 $mmol_c$ dm^{-3}; Mg= 1 $mmol_c$ dm^{-3}; sum of bases (SB)= 4 $mmol_c$ dm^{-3}; CEC= 36 $mmol_c$ dm^{-3} and base saturation (V)= 12 %. Liming was carried out 30 days before the implementation of the experiment, using calcitic limestone (PRNT = 88%), to increase base saturation to 80%. Liming, planting and cover fertilization were carried out, according to the recommendation of Bulletin 100 [12].

Cultivar Caserta was used. Sowing (September 10, 2018) was carried out in polypropylene trays of 162 cells, containing substrate for vegetables. The transplantation was done when the seedlings had the second leaf (October 01, 2018).

The experimental design used was randomized blocks, with four treatments and seven replications. The treatments were constituted by the only calcium application, only boron application, combined calcium and boron application and the control treatment (without application of calcium and/or boron). Each experimental unit was consisted by three pots containing one plant each one.

Calcium and boron were applied at a concentration of 0.2 and 0.1%, respectively. Calcium chloride, which contains 27% Ca, and boric acid, which contains 17% B, were used as the source of calcium and boron, respectively. The foliar applications were made with a costal sprayer, in the morning (between 7:30 am and 8:30 am). Applications were carried out on alternate days, starting when the first flower buds appeared (October 09, 2018), and ending when most fruits were 20 cm long.

Considering that some of the possible effects of Ca and B are the increase in the production of pollen grains, so that the effect on the production and / or quality of pollen grains is not “spread” by insects with pollination of flowers from different plots, manual pollination was performed only among flowers from the same plot, according to the methodology described by Cardoso and Souza Neto [13]. To carry out this manual pollination, the tips of the petals of the selected male and female flower buds, still closed, were tied with a red wool the afternoon before the crossing, so that the pollination could be done the next morning. Pollination was done by leaning the anthers of one male flower on the stigma of one female flower, leaving all the pollen attached. The female flowers that were open at the time of pollination and the smallest male flowers were discarded, because they were considered non-standard.

After pollination, the date of the crossing was identified with a tag tied to the peduncle of the female flower and this was protected with a paper bag. Pollinations were performed on three female flowers per plant. After fruit set, some fruits have been removed leaving only one fruit per plant for evaluating seed production per fruit without competition between fruits in the plants, which can be an important factor that interferes with seed quality.

Weed control was performed whenever it is necessary, manually. To management powdery mildew fungicide based on metiram (550 g kg^{-1}) more pyraclostrobin (50 g kg^{-1}) was used. Drip irrigation was performed twice a day (9 am and 4 pm).

Fruit harvests started when it reached the ripe stage characterized by yellowish color, followed by resting of 15 days to complete seed maturation [13]. The seed extraction was done manually, cutting the fruits in the longitudinal direction, washing the seeds on sieves with water and, then, placed on clay dishes to dry during approximately 24 hours. After this process, the seeds were placed in paper bags and stored in a dry chamber (40% RH and 20ºC), in order to uniform the water content in approximately 8%. After this period, the seeds were subjected to cleaning to remove the “empty and damaged seeds” with the aid of the seed separator by density (model 'De Leo Tipo 1'), as described by Cardoso and Pavan [14]. These classified seeds were used for production and quality assessments, discarding empty and damaged.

The following evaluations were made: a) number of pollen grains per flower, according to methodology adapted from Nogueira and coauthors [15], being evaluated five flower buds in the pre-anthesis stage per plot; b) weight of seeds per fruit: after the extraction, drying and processing of the seeds of each fruit, the weight was obtained through scale (precision of 0.0001 g); c) number of seeds per fruit: obtained by counting the seeds of each fruit; d) hundred seeds weight: eight replications of 100 seeds were sampled and the weight was evaluated using a scale (precision of 0.0001 g) [16]; e) germination: four samples of 50 seeds for each plot were used. The seeds were placed on paper towel substrate (Germitest) moistened with distilled water in a ratio of 2.5 times its dry weight. The paper rolls were kept in a germinator at 25 C, according to the methodology of the Seed Analysis Rules [16], and the number of normal seedlings was counted on the 8th days after sowing (DAS), expressed as percentage of germination; f) first germination count: the number of normal seedlings on the 4th DAS on the germination test described was used as a vigor test, expressed as a percentage; g) speed of germination index (SGI): daily observations were made after installing the germination test, counting the number of seeds germinated per day and calculating the SGI [17].

The data were submitted to Lilliefors tests, for normality, and Cochran and Bartlett tests, for homogeneity of variances and, when significant, analysis of variance was performed and and Tukey test ($p < 0.05$) was used to compare averages.

RESULTS and DISCUSSION

The isolated or combined application of calcium and boron did not affect seed weight per fruit or hundred seeds weight (Figure 1A and 1B). The observed values of hundred seeds weight (average 12.4g) are similar to those reported by Lima and coauthors [18] of 12.3g, when they used the amount of pollen equivalent to a male flower in manual pollination of one female flower, that is, the same amount used in this research and with the same cultivar Caserta. However, the seed weight per fruit (average 31 g) was greater than the average reported by Lima and coauthors [18] (21 g).

The application of B promoted a lower number of seeds per fruit, even when associated with calcium (Figure 1C). The combined application of Ca and B may have promoted antagonism between these nutrients, since the increase of Ca in plant tissue reduces the translocation of B [19], and the deficiency of B alters the translocation of Ca to the stem and fruit [20,21]. Ca isolation resulted in a greater number of seeds per fruit, when compared to treatments with B (Figure 1C). Probably, the greatest number of seeds was achieved by the greatest number of pollen grains resulting from the treatment with calcium application, which was approximately twice that of the treatment with combined application of calcium and boron (Figure 1D). In the genus *Cucurbita*, it has been reported that the greater the amount of pollen, the greater the seed production [18,22]. Nakada-Freitas and coauthors [23] related higher seed production per plant in cauliflower with application of calcium.

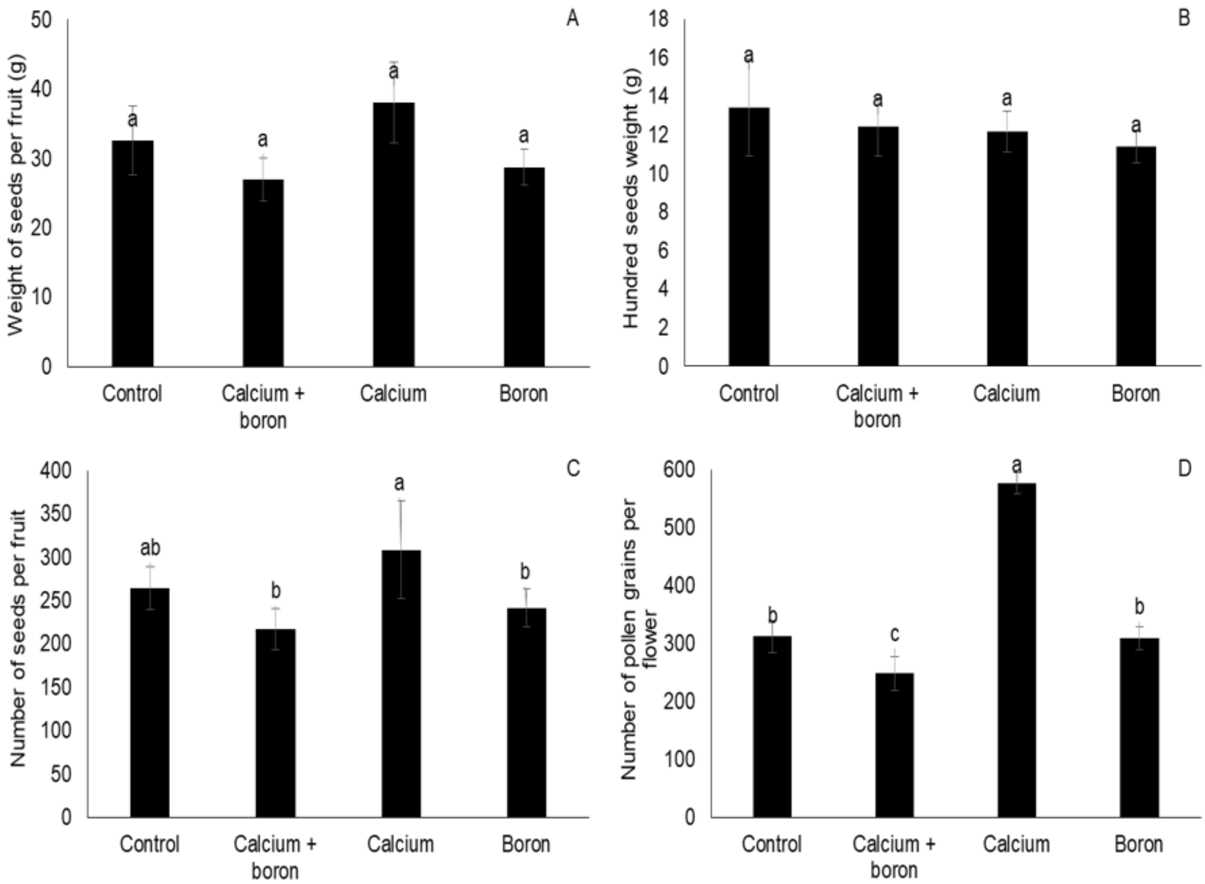

Figure 1. Weight of seeds per fruit (A), hundred seeds weight (B), number of seeds per fruit (C) and number of pollen grains per flower (D) of zucchini in function of foliar application of calcium and boron. Means followed by same letters do not differ each other by Tukey´s test (p<0,05).

Boron is indispensable during seed formation, as it acts in the process of pollen grain formation and in pollen tube growth [8]. Its deficiency leads to floral abortion and poor seed formation. However, it is the micronutrient that has the narrowest limit between deficiency and toxicity [6], which may explain the deleterious effect on the formation of pollen grains and, consequently, seeds. There are no recommendations for doses and frequency of foliar application of this micronutrient in zucchini and, probably, the dose and/or frequency used were excessive, causing toxicity. However, it is worth mentioning that the use of boron in zucchini needs further studies, considering that, perhaps, the cultivar Caserta is more sensitive than others genotypes, in addition to the dose and frequency not being adequate.

For seed germination, there were no differences among treatments, with an average of 93% (Figure 2A). The germination values obtained are much higher than the minimum required by MAPA for marketing zucchini seeds, which is 80%. However, according to Cardoso and coauthors [24], considering the competitiveness among companies in the seed market, these official standards are out of step with that practiced in the professional market, making it difficult to sell hybrid seeds with germination lesser than 90% in cucurbits. However, even with this pattern, the excellent quality of the seeds obtained is observed.

Regarding to seeds vigor, the first germination count did not show differences among treatments, with an average of 92% (Figure 2B). Already to speed of germination index (SGI), the treatment with application of calcium and boron was inferior to the control (Figure 2C), showing that the two nutrients together harmed the vigor of the seeds. On the other hand, the application of only calcium or boron did not differ from the control, showing that isolated they did not benefit or harm the vigor of the seeds.

The lowest seed quality obtained in the treatment with application of calcium and boron may be related to the smaller amount of pollen grains. Whereas Lima and coauthors [18] observed lower production and quality (germination and vigor) of seeds in 'Caserta' zucchini when using less pollen grains in manual pollination. Davis and coauthors [25] observed that pumpkin seeds produced with a larger amount of pollen grains showed faster germination.

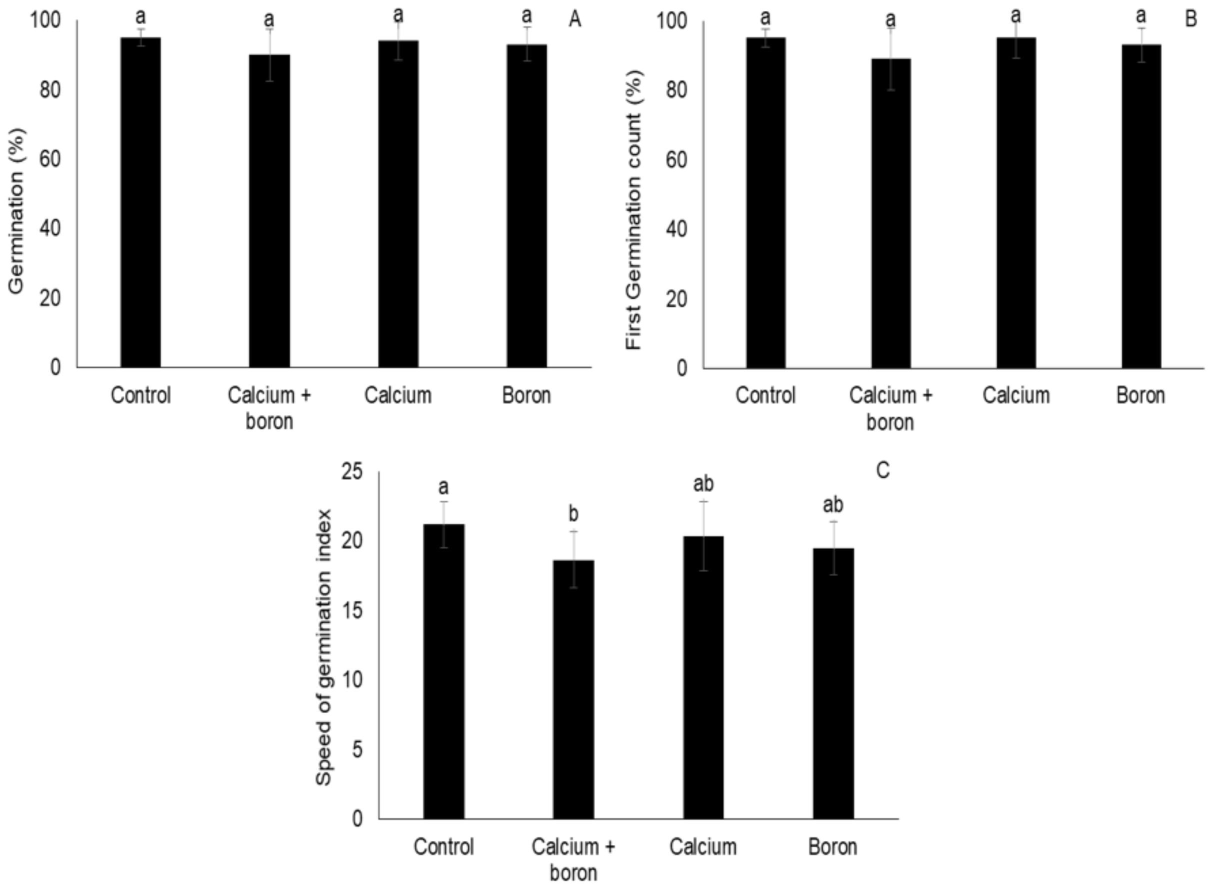

Figure 2. Germination (A), germination fist count (B) and speed of germination index (C) of zucchini seeds in function of foliar application of calcium and boron. Means followed by same letters do not differ each other by Tukey´s test (p<0,05).

Boron is very important to fruit and seed formation process, because it acts in cell walls formation, cell division, pollen grain germination, flowering and fruit formation. Its deficiency causes low fertilization of flowers, fall of flower buds and reduction in the number of fruits. In the reproductive phase, this micronutrient deficiency reduces male-fertility due to the damage to microsporogenesis and the subsequent growth of the pollen tube [26] and also causes malformation of fruits and seeds. However, it is worth mentioning that the boron has a narrow gap between deficiency and toxicity [6], which can explain the results of this research with zucchini, that is, probably toxicity due to excess boron. The foliar application of boron reduced the number of seeds produced per fruit (Figure 1) and, when applied together with calcium, in addition to reduce seed production, it also harmed vigor, assessed by the GSI (Figure 2).

Calcium, like boron, is an essential nutrient for fertilization and fixation of flowers and fruits. Calcium deficiency impairs the germination of pollen grains [27] and causes apical rot in fruits in some species, including Cucurbitas.

Despite the importance of calcium, this nutrient is a little accumulated in the seeds, a fact that it is probably related to little mobility in the plant by phloem. According to Kano and coauthors [28], its accumulation in the seeds must occur only by absorption and transport during seed maturation, without redistribution of the senescent leaves to the seeds. Lanna and coauthors [29] observed that calcium was the least accumulated macronutrient in 'Caserta' zucchini seeds. However, its application resulted in a greater quantity of pollen grains per flower (Figure 1), which may favor greater seed production, mainly in manual pollinations used both in the production of seeds of some hybrids and in breeding [13,22].

For the breeder, a greater amount of pollen in manual pollination, in addition to favoring greater seed production, may result in the indirect selection of progenies with greater productive potential. Cardoso [22] reported that after two manual crossing cycles with a greater amount of pollen grains, seeds obtained generated plants with greater productive potential. There are reports that when there is a large amount of pollen grains on the stigma, they compete with each other and the most vigorous prevail in the fertilization of ovules, resulting in more vigorous seeds. Davis and coauthors [25] and Hormaza and Herrero [30] considered that the breeder when using large quantities of pollen grains establish competition capable of selecting the most vigorous ones. Thus, for the breeder, there is the possibility of selecting more vigorous populations only by exposing the female flowers to a large amount of pollen, without the need for large areas, only taking advantage of the intense competition among pollen grains [25]. So, the foliar application of Ca can result in greater amount of pollen grains per male flower used in a manual pollination.

CONCLUSION

The foliar application of calcium and boron, in an only or combined, does not affect the seed quality of zucchini. Although, calcium increases the production of pollen grains per flower, showing to be a promise treatment for production of zucchini seeds.

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Conflicts of Interest: The authors declare no conflict of interest.

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