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Bacillus aryabhattai dose recommendation for corn seed inoculation

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Abstract: The use of plant growth-promoting bacteria (PGPB) can be a sustainable alternative to improve the uniformity and speed of emergence and initial growth of plants. However, the effects of *Bacillus aryabhattai* inoculation are still incipient and inconclusive, and there is no recommendation for the use of inoculant doses containing *B. aryabhattai*. This study was conducted to investigate the effectiveness of *B. aryabhattai* inoculation in improving the germination and initial growth of corn plants (*Zea mays* L.), and to determine the optimal inoculant dose to be recommended for inoculation of corn crops. Seeds were inoculated with 0, 10, 20, 30 and 40-mL kg^{-1} of the inoculant containing the strain CMAA 1363 of *Bacillus aryabhattai*. Four replicates of 50 seeds were sown in plastic boxes containing washed coarse sand. At 18 days after sowing, the emergence rate (E), shoot length (SL), root length (RL), shoot dry matter (SDM) and root dry matter (RDM) was recorded. The doses of inoculant containing *B. aryabhattai* applied to corn seeds did not affect the seedling emergence rate. Corn plants inoculated with *B. aryabhattai* have greater length and greater dry matter accumulation of in the shoots and roots when compared to non-inoculated plants. These results showed that the use of inoculant containing *B. aryabhattai* can be applied to improve the initial growth of corn seedlings. The optimal dose of inoculant containing *B. aryabhattai* to be applied to corn seeds can range between 20 and 22-mL kg^{-1} of inoculant.

Keywords: *Zea mays* L., plant growth-promoting bacteria, biotechnology, optimal inoculant dose.

1. Introduction

The world population is expected to reach 9.8 billion people in 2050 and, combined with issues of sustainability and climate change, has challenged modern agriculture in increasing the food supply (Zarocostas, 2022; Daszkiewicz, 2022). In this challenging agricultural scenario, the use of biotechnology can increase food production and reduce the impacts caused by global environmental change. In agriculture, biotechnology is linked to a set of techniques that use (micro)organisms to develop products and processes that are important for sustainable agricultural activity (Das et al., 2023). Recent advances in biotechnology have revolutionized the production and quality of food produced in the last decade (Steinwand, Ronald, 2020; Ranjha et al., 2022). In this context, the use of biological products plays an important role in advancing food production and protecting plants and the environment against harmful agents (Das et al., 2023).

Seed germination is the first and most sensitive and critical phase in the plant life cycle, and the rapid establishment of plants in the field has contributed to the sustainability of food production systems. Among the alternatives to improve the uniformity and speed of emergence and growth of plants in the field, the use of biological products produced from plant growth-promoting bacteria (PGPB) has stood out in recent years in Brazil (Souza et al., 2015; Al-Tammar & Khalifa, 2022; Fiodor et al., 2023). Seed inoculation with PGPB has been useful in almost all crops around the world and is an ecologically friendly alternative to the environment and production system. Many rhizobacteria have specialized mechanisms that play a key role in stimulating plant growth under various environmental conditions. The PGPB, when inoculated onto seeds, act to increase the hormonal and nutritional metabolism of plants, signal transduction and the synthesis of volatile organic compounds, which can stimulate germination and initial growth of plants, especially the root system (Numan et al., 2018; Sarkar et al., 2018; Ayaz et al., 2022).

Among plant growth-promoting bacteria with the potential to stimulate seed germination and plant growth, the genus *Bacillus* is one of the most studied groups of rhizobacteria for the inoculation of a wide range of plant species (Ferreira et al., 2018; Ansari et al., 2019; Ibarra-Villarreal et al., 2021). Indeed, Ansari et al. (2019) reported that *Bacillus pumilus* inoculation stimulated the seed germination and initial growth of wheat plants. Ferreira et al. (2018) showed that *Bacillus subtilis* inoculation improved plant growth and attenuated biochemical damage induced by salinity stress in maize plants. Upadhyay & Singh (2015) reported that inoculation of *Bacillus aquimaris* improved nutrient absorption and salinity tolerance of wheat plants. However, the effects of *Bacillus aryabatthai* inoculation on improving seed germination and plant growth are still incipient and inconclusive.

Bacillus aryabhattai is a gram-positive rhizobacteria, capable of colonizing the root system of a wide range of plant species (Ramesh et al., 2014; Ahmad et al., 2019). This rhizobacteria has enormous potential for agriculture, especially as it promotes numerous benefits for the physiological, hormonal, and nutritional metabolism of plants (Ramesh et al., 2014; Ahmad et al., 2019; Antil et al., 2021). However, there is no recommendation for the use of inoculant doses containing *Bacillus aryabhattai* strains in corn seeds.

This study aimed to evaluate the effectiveness of *Bacillus aryabhattai* inoculation in improving the germination and initial growth of corn plants (*Zea mays* L.), and to determine the optimal inoculant dose to be recommended for inoculation of corn seeds.

2. Material and Methods

2.1 Plant material and treatments

Corn seeds (*Zea mays* L., hybrid 20A38 VIP3) were surface sterilized in 2% (v/v) sodium hypochlorite solution for 5 minutes and rinsed three times in distilled water. The sterilized seeds were then dried in a laboratory environment (in the shade) for 48 hours and used in this study.

The seeds were inoculated using 0, 10-, 20-, 30- and 40-mL kg^{-1} of the commercial liquid inoculant Acta Ary® (Acta Bio, Jaboticabal, São Paulo) containing *Bacillus aryabhattai* strain CMAA 1363, with a minimum concentration of 1.0×10^9 colony-forming units per milliliter of inoculant (CFU mL^{-1}). The treatments were arranged in a completely randomized experimental design with four replications of 50 seeds.

2.2 Plant emergence and growth conditions

Four replicates of 50 seeds were sown in plastic boxes (42 × 28 × 6 cm) containing washed coarse sand, at a depth of 2.0 cm. The germination substrate (sand) was then moistened with distilled water and the plastic boxes were kept under laboratory conditions for 15 days. The temperature during the experimental bioassay varied from 21.8 °C to 29.4 °C, with an average value of 25.2 °C.

2.3 Measurement of seedling emergence and growth

At 8 days after sowing, the emergence rate (E) of corn seedlings was recorded. Seedlings were considered emerged when the epicotyl was longer than 5.0 mm. At 18 days after sowing, ten seedlings per replicate were randomly chosen to measure the length and dry matter of the shoot and roots. The shoot length (SL) and root length (RL) were determined using a ruler graduated in millimeters. The shoot dry matter (SDM) and root dry matter (RDM) was determined on an analytical balance (accuracy of ± 0.0001 g) after drying the plant material in an oven at 85 °C for 48 h.

2.4 Statistical analyses

The data was submitted to regression analysis and significant equations with the higher coefficient of determination (F test, $p < 0.05$) were adjusted. All analyses were performed using SigmaPlot 11.0 software for Windows (Systat Software, Inc., San Jose, CA, USA).

3. Results

The emergence rate of corn seedlings was not significantly influenced by the doses of inoculant containing *Bacillus aryabhattai*. The seedling emergence rate was 100%, regardless of the dose of inoculant applied to the seeds.

The doses of inoculant containing *B. aryabhattai* applied to the seeds significantly ($p < 0.05$) affected the growth of corn seedlings (Figure 1 and 2). The shoot length increased from 23.4 cm in non-inoculated seedlings to a maximum of 28.8 cm with the application of 21.2 mL kg^{-1} of inoculant containing *B. aryabhattai*, indicating an average increase of 23% (Figure 1A). The root length increased from 21.6 cm in non-inoculated seedlings to a maximum of 28.6 cm with the application of 21.7 mL kg^{-1} of inoculant containing *B. aryabhattai*, indicating an increase of 32% (Figure 1B). The total plant length increased from 45.0 cm in non-inoculated seedlings to a maximum of 57.9 cm with the application of 21.8 mL kg^{-1} of inoculant containing *B. aryabhattai*, indicating an increase of 29% (Figure 1C).

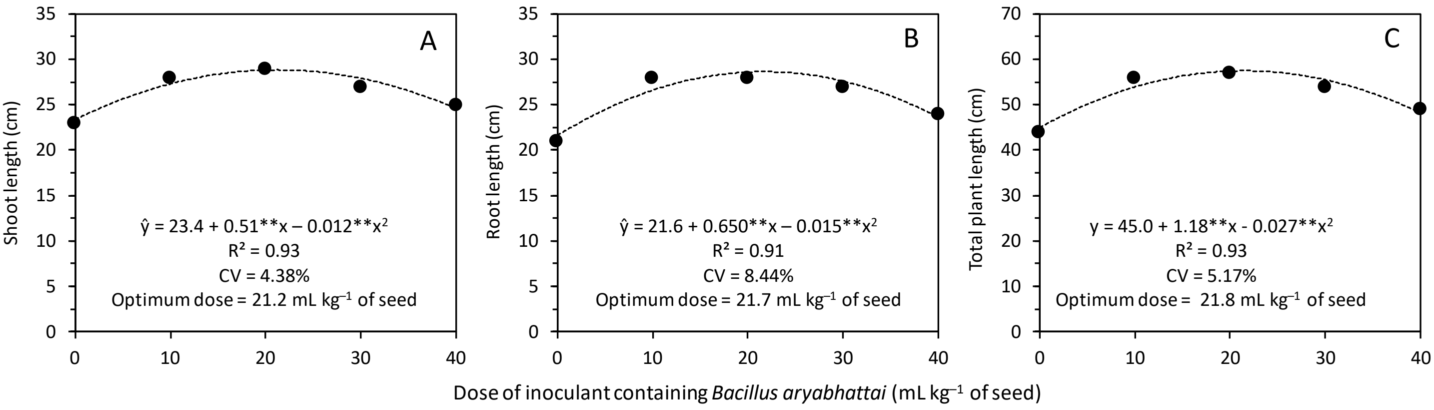

Figure 1. Effect of inoculant doses containing *Bacillus aryabhattai* CMAA 1363 applied to seeds on the shoot length (A), root length (B) and total length of corn plants (*Zea mays* L., hybrid 20A38 VIP3). **: statistical significance at 1% by t test. CV: coefficient of variation.

The dry matter production of corn seedlings was significantly ($p < 0.05$) affected by the doses of inoculant containing *B. aryabhattai* applied to the seeds. (Figure 3). The shoot dry matter increased from 76.1 mg plant^{-1} in non-inoculated seedlings to a maximum of 108.1 mg plant^{-1} with the application of 20.1 mL kg^{-1} of inoculant, indicating an average increase of 42% (Figure 3A). The root dry matter increased from 41.8 mg plant^{-1} in non-inoculated seedlings to a maximum of 64.0 mg plant^{-1} with the application of 20.5 mL kg^{-1} of inoculant, indicating an increase of 53% (Figure 3B). The total plant length increased from 118.0 mg plant^{-1} in non-inoculated seedlings to a maximum of 172.4 mg plant^{-1} with the application of 20.3 mL kg^{-1} of inoculant containing *B. aryabhattai*, indicating an average increase of 46% (Figure 3C).

Figure 2. Corn plants (*Zea mays* L., hybrid 20A38 VIP3) inoculated with different doses of inoculant containing *Bacillus aryabhattai* CMAA 1363 at 18 days after sowing. Photograph: F. Steiner (2023).

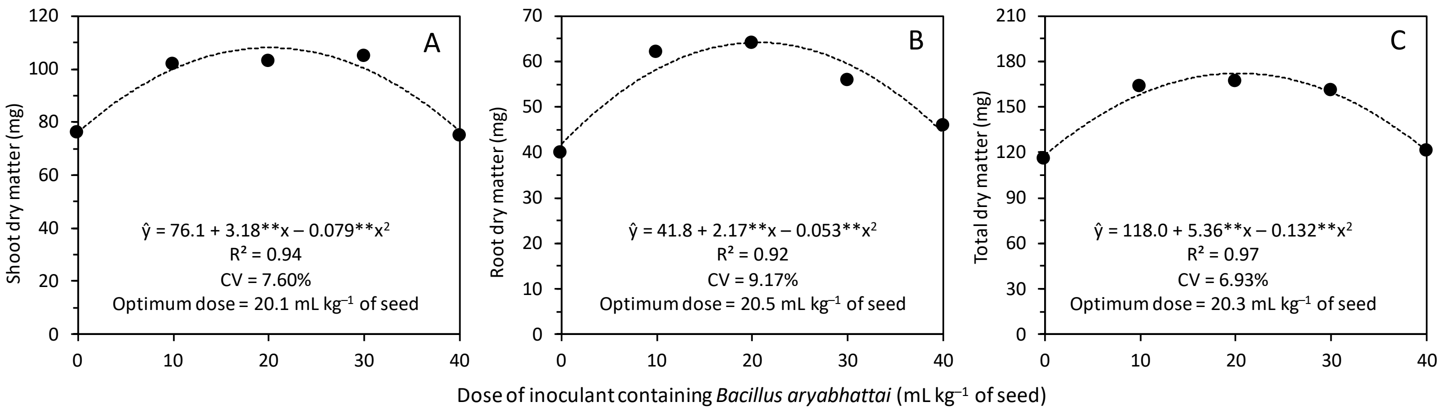

Figure 3. Effect of inoculant doses containing *Bacillus aryabhattai* CMAA 1363 applied to seeds on the shoot dry matter (A), root dry matter (B) and total dry matter of corn plants (*Zea mays* L., hybrid 20A38 VIP3). **: statistical significance at 1% by t test. CV: coefficient of variation.

4. Discussion

The application of different doses of inoculant containing *B. aryabhattai* to corn seeds did not limit the seedling emergence process. Doses of 0 to 40 mL kg^{-1} of inoculant applied to corn seeds resulted in 100% seedling emergence. These results show that the application of up to 40 mL kg^{-1} of inoculant containing *B. aryabhattai* did not have phytotoxic effects on the emergence capacity of corn plants.

Inoculation of *B. aryabhattai* improved the initial growth of corn seedlings, especially when 20 to 22 mL kg^{-1} of inoculant was applied to the seeds (Figures 1, 2 and 3). Silva & Silva (2023) showed the use of 4- and 8-mL kg^{-1} of inoculant containing *B. aryabhattai* improved seed germination and shoot growth of soybean seedlings under water stress conditions. Similar results with other *Bacillus* species are reported in the literature. Indeed, Ansari et al. (2019) reported that *Bacillus pumilus* inoculation stimulated the seed germination and initial growth of wheat plants. Ferreira et al. (2018) showed that *Bacillus subtilis* inoculation improved plant growth and attenuated biochemical damage induced by salinity stress in maize plants. Upadhyay & Singh (2015) reported that inoculation of *Bacillus aquimaris* improved nutrient absorption and salinity tolerance of wheat plants. Other studies have also proven the beneficial effects of *B. pumilus* inoculation in improving the growth of rice plants (Khan et al., 2016), of *B. paralicheniformis* inoculation in wheat plants (Ibarra-Villarreal et al., 2021) and the inoculation of *B. amyloliquefaciens* in *Arabidopsis thaliana* plants (Liu et al., 2017) and rice plants (Nautiyal et al., 2013) plants.

The inoculation with *B. aryabhattai* CMAA 1363 resulted in the higher dry matter accumulation of shoots and roots compared to non-inoculated plants (Figure 3). These results indicate that the *B. aryabhattai* inoculation in corn seeds improved the growth and dry matter production of the plants, especially the root system (Figure 2). This rhizobacteria may promote plant growth through the several mechanisms, such as the regulation and synthesis of plant hormones, mainly auxin (AX), abscisic acid (ABA) and jasmonic acid (JA) (Ramesh et al., 2014; Ahmad et al., 2019). Plants inoculated with PGPB producing indole-3-acetic acid (IAA) - an auxin, such as *Bacillus aryabhattai*, have an increase in the formation of lateral roots and root hairs and/or higher root growth, increasing water and nutrient uptake, which can help plants to overcome environmental stresses and improving the dry matter accumulation of the plants (Ramesh et al., 2014; Ahmad et al., 2019; Antil et al., 2021).

Other beneficial effects of *B. aryabhattai* inoculation in improving plant growth are associated with increased activity of biochemical metabolism, increased tolerance of plants to abiotic and biotic stresses, and production of organic acids such as lactic acid, citric acid, acid gluconic and malonic acid, and growth regulators, such as siderophores, which are capable of fixing nitrogen and solubilizing zinc and phosphate (Ramesh et al., 2014; Ahmad et al., 2019; Antil et al., 2021). Therefore, the association of all these beneficial effects on the physiological, hormonal and nutritional metabolism of plants makes *B. aryabhattai* an excellent option for the production of microbiological inoculants with enormous potential for use in Brazilian and global agriculture (Ramesh et al., 2014; Ahmad et al., 2019; Antil et al., 2021).

5. Conclusions

The use of inoculant containing *Bacillus aryabhattai* improved the initial growth and dry matter production of corn plants and had no negative impact on the seedling emergence rate.

The optimal dose of inoculant containing *Bacillus aryabhattai* to be applied to corn seeds can range between 20- and 22-mL kg^{-1} of inoculant.

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7. Additional Information

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7.3 Interest conflicts

We declare there is no conflict of interest.